THE UTILIZATION OF ENERGY PRODUCING NUTRIMENT AND PROTEIN AS AFFECTED BY INDIVIDUAL NUTRIENT DEFICIENCIES

III. THE EFFECTS OF THE PLANE OF PROTEIN INTAKE [1]

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SEVEN FIGURES

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Two similar experiments were conducted on the plane of protein intake as affecting the utilization of food energy and protein by albino rats, this study being one of a series performed by the same original routine, especially from the point of view of an interest in measures of the nutritive values of foods.

Other studies on related subjects by the same routine have been published by Swift, Kahlenberg, Voris and Forbes ('34), McClure, Voris and Forbes ('34), and Braman, Black, Kahlenberg, Voris, Swift and Forbes ('35).

The first of the two experiments to be discussed was conducted in 1933–1934. The results appeared to be valid, but were in important details unexpected. In order to confirm or to disprove these findings, therefore, and to throw light upon the comparatively new experimental procedure employed, this first experiment was repeated, as nearly as practicable in the same way, in the years 1934–1935.

In each of these experiments a study was made, with forty-eight albino rats as subjects, of planes of protein intake varying between moderate deficiency and approximate optimum.

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Four diets were compounded to contain 10, 15, 20 and 25 per cent of protein, respectively, to have exactly the same gross energy value, and to supply all known mineral and vitamin requirements. These four diets were fed one to each rat of groups of four individuals, each such group being of one sex and of the same litter, and of approximately the same initial live weight. The rats were fed in accord with the principle of paired feeding (in this case quadruplets instead of pairs); each rat of a group of four receiving the same energy but different protein intake; there being, in each experiment, twelve individuals on each of the four treatments.

The diets were composed as follows:

First experiment; constant constituents: butter fat 10 per cent, brewer's yeast 5 per cent, irradiated yeast 1 per cent, Cellu flour 4 per cent, Osborne and Mendel salt mixture 4 per cent and sodium chloride 1 per cent; and varied constituents, dextrin 51.156 to 62.325 per cent, casein 7.573 to 23.844 per cent and Crisco 0 to 5.102 per cent.

Second experiment; constant constituents: butter fat 10 per cent, brewer's yeast 5.25 per cent, irradiated yeast 0.75 per cent, Cellu flour 4.0 per cent, Osborne and Mendel salt mixture 4.0 per cent and sodium chloride 1.0 per cent; and of the varied constituents the percentages were of dextrin 51.156 to 62.325 per cent, casein 7.573 to 23.844 per cent, and Crisco 0 to 5.102 per cent.

The experimental feeding began as soon as the rats were weaned, and continued for 10 weeks.

The quantity of food given each day to each unit of four rats was determined by the quantity consumed by the individual, among the four, which ate the least. In the first experiment the total numbers of refusals of the 10, 15, 20 and 25 per cent protein rations were 213, 52, 10 and 56, respectively; and in the second experiment the numbers of refusals were 154, 97, 76 and 42, respectively. In both experiments, therefore, the 10 per cent protein diet determined the food intake for all groups.

Urine and feces were collected separately and continuously, during the 10-week period of experimentation, and were subjected to chemical analysis.

At the end of the experiments all rats were killed and analyzed, and gains of constituents were determined by comparison of data from these rats which were fed and killed with data from a control group of twelve rats killed at the beginning of the experiments.

The experiments progressed from beginning to end with unusual regularity and consistency.

TABLE 1

Average gain in body weight[1] related to dry matter of feed

PLANE OF PROTEIN INTAKE	GAIN IN BODY WEIGHT	FOOD EATEN (DRY MATTER)	DRY MATTER OF FOOD PER GRAM BODY GAIN
	gm.	*gm.*	*gm.*
Experiment no. 1			
10% protein	78.93	437.6	5.5 ± 0.12
15% protein	107.04	437.9	4.1 ± 0.11
20% protein	119.13	438.3	3.7 ± 0.09
25% protein	119.41	438.5	3.7 ± 0.09
Experiment no. 2			
10% protein	66.04	419.7	6.4 ± 0.14
15% protein	92.85	420.9	4.5 ± 0.10
20% protein	99.55	420.4	4.2 ± 0.11
25% protein	100.35	419.2	4.2 ± 0.10

[1] Contents of alimentary tract removed.

NOTE: Each datum is an average value representing twelve animals on a continuous metabolism investigation during 10 weeks.

In the course of the second experiment there was observed, in three rats among the forty-eight, slight evidence of a dietary deficiency which, however, was not sufficiently marked appreciably to affect the appetite or the growth of the rats, and was not positively diagnosed.

Rats nos. 1 and 25, on the 10 per cent protein diet, and no. 18 on the 15 per cent protein diet, exhibited loss of hair, in differing degrees, from the ventral side of the body.

On this account, and with the thought that the symptom noted might possibly be due to a fatty acid deficiency, each

rat of the forty-eight was given 3 drops of cod liver oil per day from the time this disorder was observed until the end of the experiment.

This treatment led to marked improvement of the affected rats, with respect to this condition, and the cod liver oil was accounted for as a part of the energy intake.

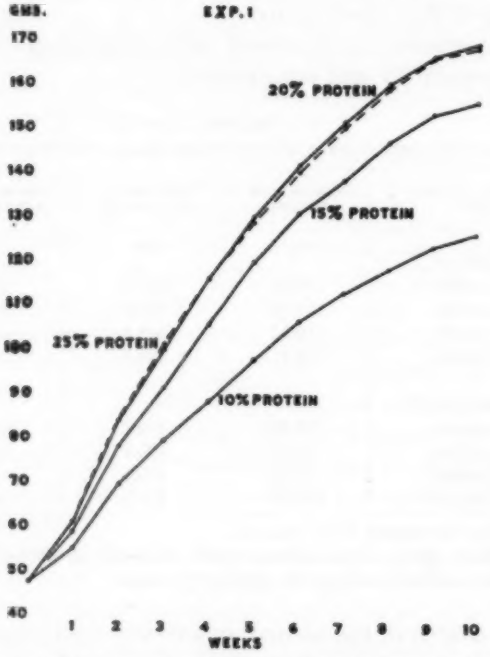

Fig. 1 Experiment 1. Average growth of albino rats during 10 weeks with the same energy but different protein intake.

Referring to table 1, and figures 1 and 2, it is clear that, with constant energy intake, the increase in percentage of protein led to greater increase in body weight, the increment in percentage of protein from 10 to 15 leading to greater increase in rate of body gain than the increment from 15 to 20 per cent, while the effects of the increment from 20 to 25

per cent of protein were so small as not to be statistically significant.

Since the energy intake remained constant the observed increase in rate of gain in body weight necessarily implied

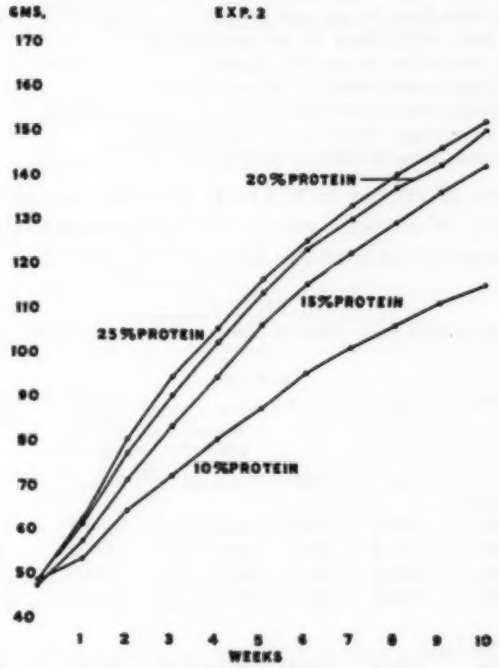

Fig. 2 Experiment 2. Average growth of albino rats during 10 weeks with the same energy but different protein intake.

a decrease in dry matter of food utilized per unit of body gain.

The rising level of protein intake from 10 to 25 per cent, therefore, brought about a progressive improvement of nutritive balance, at a diminishing rate of such improvement in relation to the increase in protein of the food.

The odds expressing the degree of significance of the gains in body weight, computed by the modification by Love ('24) of the method of 'Student' ('08), were as follows:

	Experiment no.	
	I	*II*
Gain in body weight from 15 per cent protein greater than from 10 per cent protein	10,000:1	10,000:1
Gain in body weight from 20 per cent protein greater than from 15 per cent protein	66:1	207:1
Gain in body weight from 25 per cent protein greater than from 15 per cent protein	400:1	490:1
Gain in body weight from 25 per cent protein greater than from 20 per cent protein	1:1	2:1

The gains of energy in the body growth, and the relations of the energy of the body gain to the energy and to the metabolizable energy of the food are indicated in table 2.

TABLE 2

Average energy of body gain related to energy and to metabolizable energy of food

PLANE OF PROTEIN INTAKE	FEED ENERGY	BODY GAIN		METABOLIZABLE ENERGY	BODY GAIN AS PERCENTAGE OF METABOLIZABLE ENERGY
			Per cent of feed energy		
	Cal.	*Cal.*	*per cent*	*Cal.*	*per cent*
Experiment no. 1					
10% protein	2163.6	224.2	10.4 ± 0.32	1932.7	11.6 ± 0.35
15% protein	2163.6	262.7	12.1 ± 0.50	1930.9	13.5 ± 0.53
20% protein	2163.6	295.9	13.7 ± 0.47	1938.6	15.3 ± 0.52
25% protein	2163.6	301.6	13.9 ± 0.43	1927.8	15.6 ± 0.48
Experiment no. 2					
10% protein	2075.9	226.4	10.9 ± 0.36	1858.3	12.2 ± 0.41
15% protein	2075.9	259.4	12.4 ± 0.42	1859.9	13.8 ± 0.47
20% protein	2075.9	267.0	12.9 ± 0.35	1857.4	14.4 ± 0.40
25% protein	2075.9	270.2	12.9 ± 0.42	1847.9	14.5 ± 0.47

NOTE: Each datum is an average value representing twelve animals on a continuous metabolism investigation during 10 weeks.

These data, and figures 3 and 4, show that with the same food energy intake the increase in the protein of the diets from 10 to 15 per cent, and from 15 to 20 per cent, brought about increased storage of food energy (figs. 3 and 4), and increased percentage of food energy and of metabolizable energy, as body gain (table 2); but that the increase in protein

of the diet from 20 to 25 per cent produced very little added energy retention.

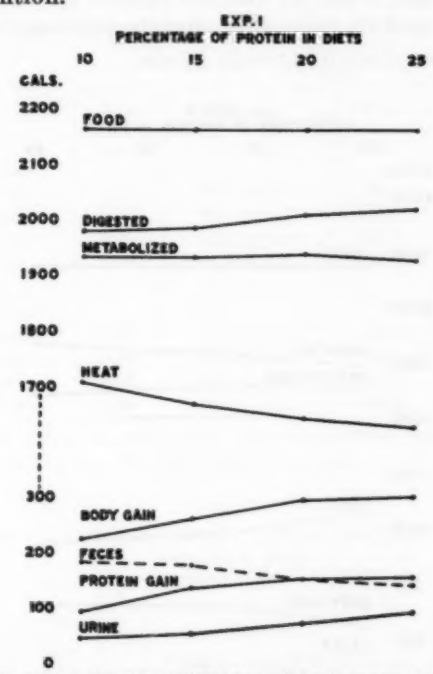

Fig. 3 Experiment 1. Average utilization of food energy during 10 weeks as affected by differences in the plane of protein intake.

The odds relating to energy gain as affected by the percentage of protein in the diets were as follows:

	Experiment no.	
	I	*II*
Energy gain from 15 per cent protein greater than from 10 per cent protein	27:1	49:1
Energy gain from 20 per cent protein greater than from 10 per cent protein	3332:1	1110:1
Energy gain from 25 per cent protein greater than from 10 per cent protein	.....	88:1
Energy gain from 20 per cent protein greater than from 15 per cent protein	18:1	2:1
Energy gain from 25 per cent protein greater than from 15 per cent protein	53:1	3:1
Energy gain from 25 per cent protein greater than from 20 per cent protein	2:1	2:1

The digestibility of both the energy producing nutrients (table 3 and figs. 3 and 4) and the protein of the diets (table 3 and figs. 5 and 6) increased slightly but consistently with increase in the plane of protein intake.

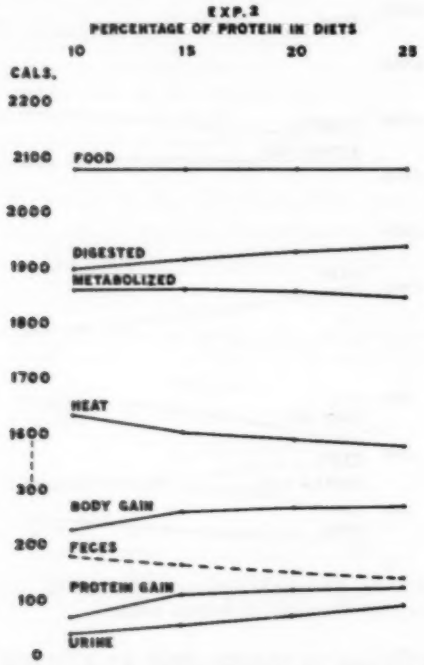

Fig. 4 Experiment 2. Average utilization of food energy during 10 weeks as affected by differences in the plane of protein intake.

The metabolizable energy of the diets, however, was essentially the same at all planes of protein intake (figs. 3 and 4), since the urinary energy increased almost exactly in proportion as the feces energy decreased, with rise in proportion of protein in the diets.

Data relating to the effects of the plane of protein intake on the composition of the body gain are given in table 4.

TABLE 3

Average digestibility of nitrogen and energy producing nutriment

PLANE OF PROTEIN INTAKE	NITROGEN		ENERGY	
	Feces	Digested	Feces	Digested
	gm.	*per cent*	*Cal.*	*per cent*
Experiment no. 1				
10% protein	0.88	88.1 ± 0.24	184.4	91.5 ± 0.17
15% protein	0.90	91.8 ± 0.13	178.0	91.8 ± 0.26
20% protein	0.97	93.3 ± 0.10	152.7	92.9 ± 0.07
25% protein	1.05	94.2 ± 0.16	143.0	93.4 ± 0.09
Experiment no. 2				
10% protein	0.83	88.3 ± 0.14	178.4	91.4 ± 0.13
15% protein	0.90	91.5 ± 0.12	162.0	92.2 ± 0.12
20% protein	0.95	93.2 ± 0.08	147.7	92.9 ± 0.06
25% protein	0.96	94.6 ± 0.13	138.2	93.3 ± 0.06

NOTE: Each datum is an average value representing twelve animals on a continuous metabolism investigation during 10 weeks.

TABLE 4

Average nitrogen of body gain related to fat and energy of body gain and to nitrogen of feed

PLANE OF PROTEIN INTAKE	NITROGEN OF BODY GAIN	FAT GAINED		ENERGY GAINED			NITROGEN OF FEED	
			Per gram nitrogen gained	Total	As protein	As fat		Utilized for body gain
	gm.	*gm.*	*gm.*	*Cal.*	*Cal.*	*Cal.*	*gm.*	*per cent*
Experiment no. 1								
10% protein	2.51	13.9	5.5 ± 0.18	224.2	93.4	130.8	7.41	33.9 ± 0.58
15% protein	3.68	13.3	3.6 ± 0.26	262.7	137.4	125.3	11.04	33.3 ± 0.63
20% protein	4.13	15.2	3.7 ± 0.24	295.9	152.6	143.3	14.49	28.5 ± 0.62
25% protein	4.17	15.4	3.7 ± 0.25	301.6	156.4	145.2	18.17	22.9 ± 0.60
Experiment no. 2								
10% protein	1.91	16.8	8.8 ± 0.45	226.4	68.4	158.0	7.05	27.1 ± 0.57
15% protein	3.02	16.1	5.3 ± 0.33	259.4	107.7	151.7	10.53	28.7 ± 0.58
20% protein	3.29	15.9	4.8 ± 0.34	267.0	117.2	149.8	14.06	23.6 ± 0.68
25% protein	3.42	15.7	4.6 ± 0.35	270.2	121.5	148.7	17.59	19.4 ± 0.60

NOTE: Each datum is an average value representing twelve animals on a continuous metabolism investigation during 10 weeks.

With increase in the proportion of protein in the diets there were invariably increases, in the same order, in the nitrogen and energy gained, and usually a decrease in the percentage of food nitrogen utilized for body gain—signifying diminishing necessity for economy in nitrogen utilization.

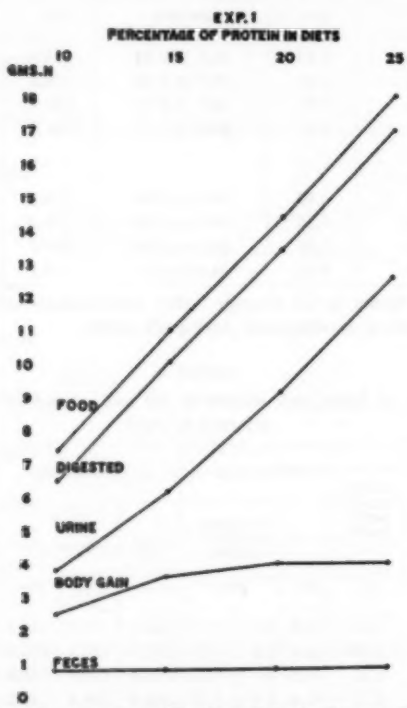

Fig. 5 Experiment 1. Average utilization of food nitrogen during 10 weeks as affected by differences in the plane of protein intake.

This situation is graphically represented in figures 5 and 6 by the curves showing that with increase in the protein contents of the diets the nitrogen retained as body gain increased at a decreasing rate, and the nitrogen of the urine increased at an increasing rate.

The curves representing feces nitrogen, in figures 5 and 6, show that with increase in the protein of the diets the feces nitrogen increased but little, because of the fact that the protein of the diets was mainly in the form of casein, which was highly digestible.

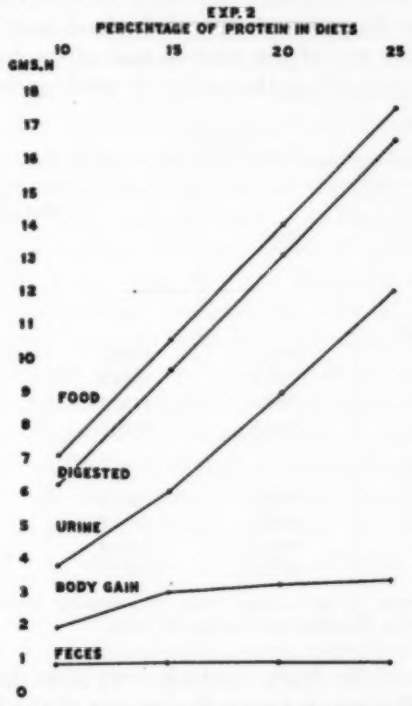

Fig. 6 Experiment 2. Average utilization of food nitrogen during 10 weeks as affected by differences in the plane of protein intake.

The relation of the protein content of the diets to the heat loss is shown in table 5, and in figures 3 and 4.

The first thought that naturally comes to mind in this relation is that with increase in the protein content of these equicaloric diets the specific dynamic effect of the protein would have the effect to increase the heat production. This, however, is incorrect. The results of these experiments show

clearly that increases in the protein content of equicaloric diets which improve their nutritive balance, as indicated by increased retention of protein and energy, and increased gain in body weight, have the effect to diminish rather than to increase the heat production.

The heat production was measured indirectly, as the difference between the gross energy of the food and the combined values for the energy of the excreta and of the body increase, in one value representing the entire 10-week period of experimentation.

TABLE 5

Average heat loss related to energy of food

PLANE OF PROTEIN INTAKE	FEED ENERGY	HEAT LOSS	
			Per cent of feed energy
	Cal.	*Cal.*	*per cent*
Experiment no. 1			
10% protein	2163.6	1708.5	79.0 ± 0.31
15% protein	2163.6	1668.2	77.1 ± 0.42
20% protein	2163.6	1642.7	75.9 ± 0.46
25% protein	2163.6	1626.2	75.2 ± 0.43
Experiment no. 2			
10% protein	2075.9	1631.9	78.6 ± 0.41
15% protein	2075.9	1600.6	77.2 ± 0.41
20% protein	2075.9	1590.4	76.6 ± 0.40
25% protein	2075.9	1577.7	76.1 ± 0.42

NOTE: Each datum is an average value representing twelve animals on a continuous metabolism investigation during 10 weeks.

The energy of the body increase was also determined indirectly, by difference between the energy of the bodies of the rats at the end of the experiment and of a control group which was carefully selected to represent the experimental groups at the beginning of the study.

In spite of the fact that this measurement of the heat production was indirect, and determined by difference, as explained, it is the judgment of the writers that the values so determined are more accurately representative of the heat production of the animals than any measurement covering only a few minutes or a few hours could possibly be.

These indirect heat measurements are relatively large values, in comparison with those representing the excreta and the body gain, and inasmuch as they represent the total heat production for a 10-week period, they should be free from such extensive errors as would be inevitable if the heat were determined directly, in short periods of observation, and were then multiplied by the large numbers required to render them representative of the whole period of experimentation.

The reliability of these indirect heat measurements is strikingly shown by the close agreement of the curves of heat production for the two experiments (figs. 3 and 4).

As an indication of the accuracy of the operations involved in this indirect heat measurement—in the first experiment the average percentage recovery of feed nitrogen, in feces, urine and body gain—was 98.3 per cent, for the forty-eight rats, with a coefficient of variation of only 1.51 per cent.

The difference between 98.3 per cent and 100 per cent recovery of nitrogen represents experimental error, including shed hair, and ammonia vaporized from the excreta.

A noteworthy arithmetic consequence of the method used of accounting for the food energy is that with equicaloric diets of essentially the same metabolizability, as in these experiments, the energy of the body gain and the heat production can only vary reciprocally—because, jointly, they complete the total.

Duplicate determinations of the energy of materials, by means of the bomb calorimeter, were accepted only when the variation was within 1 per cent, except with urine, in which case a 2 per cent variation was accepted. The duplicate analyses of the forty-eight samples of feces differed, on the average, by 0.30 per cent, and the corresponding values for ether extracts of the rat bodies differed by 0.25 per cent.

The average of the duplicate energy determinations entering into the measurement of the heat production, therefore, are believed to be not more than $\frac{1}{2}$ of 1 per cent in error.

The odds relating to the heat production as affected by the percentage of protein in the diets were as follows:

	Experiment no. I	II
Heat production from 10 per cent protein greater than from 15 per cent protein	48:1	26:1
Heat production from 10 per cent protein greater than from 20 per cent protein	1110:1	3330:1
Heat production from 15 per cent protein greater than from 20 per cent protein	19:1	2:1
Heat production from 15 per cent protein greater than from 25 per cent protein	224:1	13:1
Heat production from 20 per cent protein greater than from 25 per cent protein	4:1	4:1

A detail of importance in the conduct of this investigation was the method of control of food consumption—especially the prevention of waste of food and of contamination of excreta by waste food.

This was accomplished by means of a holder for the feed cup, made of galvanized iron and illustrated in figure 7. This apparatus, which was devised by R. W. Swift, is 5 inches wide and 3½ inches from front to back, the back being 3⅜ inches high, and the front $1\frac{3}{16}$ inch high. The feed cup itself was a 50 cc. beaker.

The device was designed for use in the cylindrical cage illustrated in this journal, vol. 8, August, 1934, p. 206. This illustration, however, does not show the inverted pie-tin which serves as a cover for the cage.

To save floor space within the cage the feeder is elevated by suspension from the top of the cage, by means of the hook shown in the figure.

The use of a beaker as a feed container makes for convenience in weighing, handling and cleaning.

The beaker is held firmly in place in a metal cup, and the surrounding waste pan catches food thrown out of the beaker. Even during long periods of use, the amount of feed thus scattered is negligible. A loose metal ring, with cross wires, is sometimes placed on top of the feed in the beaker, to prevent the rat from scooping out the feed, but the use of this accessory is rarely required.

The top of the feeder is covered by a loose piece of $\frac{1}{2}$-inch-mesh galvanized wire screen, hinged at the back, and with an opening at the center to permit access of the rat to the beaker. In cutting this opening the wires at the back are bent down, to the edge of the beaker, and the wires at the sides are bent vertically upright to discourage the rat from sleeping on this screen. If the rats learn to raise this hinged screen it may be fastened down with a wire hook.

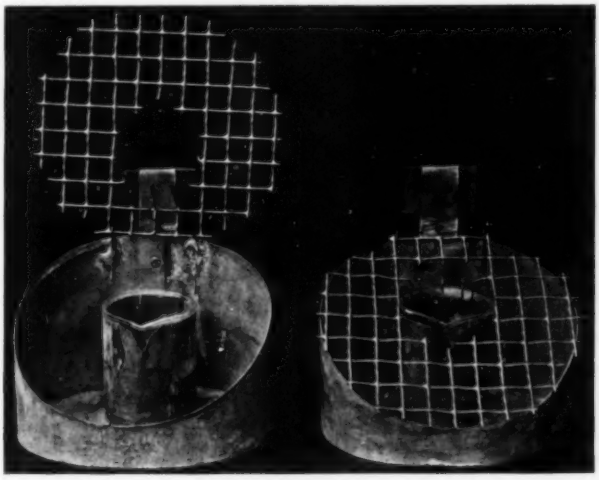

Fig. 7 Feeding cup for use in nutrition experiments with the rat.

The purpose of this screen cover is to prevent the access of the rat to feces dropped in the waste pan surrounding the beaker, though in most cases no feces are dropped there.

When this device is used with very unpalatable rations small quantities of food may be thrown outside the apparatus, but this happens much less frequently than in the use of several other types of feeders with which it has been compared.

The method of the investigation did not provide for the complete factoring of the heat production. Under the conditions existing, however, it was practicable to make a series

of heat measurements of each of the forty-eight rats utilized in the second experiment, which may be considered in the general light of measurements of basal metabolism, though basal conditions as usually defined were not maintained with exactness.

The rats being fed once per day, one measurement of the approximate basal heat production of each individual was made by the open-train Haldane procedure. Thirty-six of these measurements were made during the seventeenth to twenty-third hours and twelve during the twenty-fourth to thirtieth hours after feeding. In each case the rat was in the chamber for 7 hours, but the heat production of the first hour was rejected on account of voluntary movements of the animals when first put into the chamber.

The time at which the rats finished eating was not observed. These measurements, therefore, do not exactly represent the basal metabolism, as usually defined, though they do so in an approximate manner.

Thirty-two of the rats were subjected to this measurement during the seventh week of experimentation, and the remaining sixteen during the ninth week. The quadruplets were handled as nearly alike as possible, two animals on one day, and the other two on the following day.

The procedure followed was the same as that described by Forbes, Kriss and Miller ('34) except that the water bath was operated as an air bath, in order to avoid the necessity of drying the respiration chamber (bottle) before weighing. With this change, the rat, which apparently slept during the 6-hour respiration measurement, under the influence of the bright light, usually remained asleep while it was weighed at the end of this period.

These approximate basal heat determinations, given in table 6, show that the plane of protein intake was without appreciable effect on this measurement. The difference in the heat production of the four groups of rats, resulting from the difference in protein intake and as measured for the 10-week period, therefore, represented either physical activity, or dynamic effect, or both.

A computation of the greatest possible differences in the dynamic effects of the diets employed which might be expected in the light of the separate determination of the specific dynamic effects of protein, carbohydrate and fat, by Kriss, Forbes and Miller ('34) indicates that in all probability these differences are so small as to lie within the range of the inevitable errors of the most accurate measurements of such values that are now attainable. It is the judgment of the writers, therefore, that the differences in heat production of the rats in these experiments which are responsible for the falling curves of heat production coincident with increase in

TABLE 6

The approximate basal heat production of albino rats which received equicaloric diets containing different percentages of protein

PROTEIN IN DIET	BASAL HEAT PER HOUR	LIVE WEIGHT	SURFACE AREA	BASAL HEAT PER SQUARE CENTIMETER PER HOUR	NON-PROTEIN R. Q.
per cent	*Cal.*	*gm.*	*sq.cm.*	*Cal.*	
10	593.0	99.8	198.5	3.0	0.74
15	658.6	121.3	223.1	3.0	0.75
20	701.8	132.2	235.0	3.0	0.75
25	690.1	135.0	238.0	2.9	0.76

NOTE: Each datum is an average value representing twelve animals.

protein intake are primarily and almost exclusively expressions of differences in voluntary activity—for which there are no accurate quantitative measures, separate from the dynamic effects of the diets.

Surface area, as given in table 6, was computed by the use of the formula—surface area, in sq.cm., $= 12.54$ W (gms.)$^{0.6}$, Lee ('29).

With diets of the same energy value, therefore, differences in the plane of protein intake influence the food utilization of the growing rat in a complication of ways including, most notably, the amount and composition of the body increase, the heat loss and the efficiency with which both food energy and food nitrogen were digested and were retained as growth.

The degrees of the observed effects obviously apply with exactness only to growing rats, on the particular diets fed; but other animals, on other diets, undoubtedly respond in similar ways to similar influences.

SUMMARY

The effects of four planes of protein intake (10, 15, 20 and 25 per cent) were studied by means of two 10 weeks' growth, metabolism and body analysis experiments on forty-eight albino rats each. The rats for each experiment were selected as twelve groups of four individuals, each such group being of one sex and of the same litter, and each rat in each such group received the same energy but different protein intake. There were, therefore, twelve rats on each treatment in each experiment.

The effects of the increasing protein content of the equicaloric diets were as follows:

Increase in gain in body weight, at decreased cost in terms of dry matter of food; increase in efficiency of digestion and retention of protein and of energy-producing nutriment; increase in urinary nitrogen at an increasing rate, and increase in protein of the body at a decreasing rate; increase in energy of the urine coincident with decrease in the energy of the feces, the metabolizable energy, therefore, remaining practically constant; diminishd efficiency in the utilization of food nitrogen; no regular change in amount of fat gained, but usually a decrease in fat gained in proportion to protein gained.

Increases in the protein of equicaloric diets having the effect to improve their nutritive balance made no change in the basal heat production per unit of computed surface area, but diminished the total heat production of the animals, as they lived under normal conditions of freedom of activity.

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PATHOLOGICAL SKIN CHANGES IN THE TAIL OF THE ALBINO RAT ON A DIET DEFICIENT IN VITAMIN G [1, 2]

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SIX FIGURES

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In the course of certain vitamin G studies it was noticed by one of us (Smith, '32) that the sebaceous glands in the tails of the deficient rats were atrophied. We have now been able to confirm these findings and to show that the change is independent of the initial age of the rat and the season of the year. The sebaceous gland change is regularly accompanied by what appears to be altered secretion which is interesting in view of the fact that abnormal secretion has been reported in cases of human pellagra (Corkill, '34).

EXPERIMENTAL

Animals. White albino rats of The Wistar Institute strain were used in this work with the exception of thirteen of the controls used in experiment I. The latter were obtained locally and were of unknown, but probably poor nutritional background. Approximately equal numbers of males and females were used, and their weights were recorded weekly.

[1] Vitamin G(B_2) as used in this paper includes the factors of the vitamin B complex which withstand autoclaving.

[2] Part of the expenses of this work was covered by a grant from the Lederle Laboratories, Pearl River, New York.

The rats were divided into groups of six in cages with raised screen bottoms (1.25 cm. mesh) to minimize the effect of coprophagy. They had access to the diet and to fresh distilled water at all times.

Diet I. This is a stock diet which has proven biologically adequate in our colony. It consists of 'Bal Ra,' a commercial dog food, supplemented with cod liver oil at a level of 2 per cent and brewer's yeast at a level of 10 per cent.

Diet II. This diet was designed to approximate the one on which the 'poor whites' in the south develop pellagra and is similar to the one on which Goldberger ('15) produced pellagra in human subjects in his prison farm experiment. Its composition is as follows: corn meal (white, water ground) 50 per cent; pork fat (or salt pork) 20 per cent; Brer Rabbit molasses (Gold Label) 15 per cent; white wheat flour 10 per cent; cane sugar 5 per cent.

Diet III. This is a vitamin G deficient diet of Bourquin and Sherman ('31). It consists of purified casein 18 per cent; butter fat 8 per cent; Osborne and Mendel's ('13) salt mixture 4 per cent; cod liver oil 2 per cent; cornstarch 68 per cent. A part of the cornstarch carries the strong 80 per cent alcoholic extract from 50 gm. of whole wheat for each 100 gm. of diet as the source of B in the diet.

Diet IV. This is identical with diet III except that yeast autoclaved in thin layers 2½ hours at 15 pounds pressure is introduced at a level of 10 per cent, replacing an equal weight of cornstarch.

Diet V. This is the same as diet III except that dried egg white is introduced at a level of 10 per cent, replacing an equal weight of cornstarch (Chick and Roscoe, '29).

The egg white used as a supplement in diet V was coagulated by boiling for 30 minutes in an aluminum double boiler stirring constantly to break up the particles. The moist coagulated product was then dried in thin layers on glass dishes at a temperature from 80 to 90°C. for 2 hours and was allowed to stand over night in the oven.

Method of sectioning tail. For biopsy a portion of the tail approximately 0.5 cm. long was cut from the distal end with a very sharp razor blade and fixed in 10 per cent formalin. On necropsy three sections were made, one from the tip, one from the mid-portion, and one from the base and similarly fixed. The sections were decalcified in equal parts of 85 per cent formic acid and 20 per cent sodium citrate, embedded in paraffin and then sectioned and stained with hematoxylin and eosin.

Table 1 shows the general plan of the experiment. A biopsy was made whenever the diet was changed. Sections were also made when the animal died or was sacrificed.

Results. a. Clinical. Almost immediately after placing the animals on the deficient diets there was the usual slowing of the growth rate as illustrated for experiment III in figure 1. The life spans in experiment I varied from 70 to more than 225 days except for diet II where all were dead in 175 days. This could not be observed in the other experiments because the animals were sacrificed too soon.

Tail lesions appeared in 70 to 90 days on diets II and III. These usually consisted of a dry scaliness beginning at the tip and extending up the tail together with a dry yellow waxy coating resembling hardened sebaceous secretion (fig. 2). With the older animals of experiment I and particularly on diet II the lesions differed somewhat in being darker and more greasy, possibly contaminated with food and dirt over the longer period of time. This did not occur on the adequate diet I and disappeared gradually when autoclaved yeast was fed, the tail finally becoming quite clean and exhibiting a pink appearance as of new tissue.

One of the first symptoms observed was a staining of the wrists and forepaws with a dark red material giving a positive guaiac and benzidine test for blood. This staining had a tendency to come and go, but it appeared at sometime in all the deficient rats except one. Priapism also occurred very frequently. Reproduction did not occur on any of the deficient diets. General alopecia was fairly constant and was manifested by the disappearance of the long coarse hairs so that

TABLE 1

EXPERIMENT	SEASON	GROUPS	NUMBER OF RATS	DIET	AGE AT START OF EXPERIMENT	INITIAL WEIGHT	MAXIMUM NUMBER OF DAYS ON DEFICIENT DIET	NUMBER OF SURVIVORS AT END OF PERIOD	FATE AT THIS TIME
					days				
I	Oct. 7, 1931 to Sept. 7, 1932	I	19	I	45	74	226	3 (others killed at intervals for comparison)	Killed
		II	17	II				0	.....
		III	18	III				6	Diet IV. 1 died 1 day later. 1 died 6 days later 4 continued 36 days
II	July 19, 1933 to Dec. 5, 1933	I	6	III	23	38	112	4	2 killed 1 died 1 diet IV 36 days.
		II	2	VI				1	Killed
III	May 1, 1934 to Sept. 1, 1934	I	10	III	27–30	56	70	10	Killed
		II	15	III			90	15	Killed
		III	15	III			100	15	Killed
		IV	4	III			84	4	Diet IV (6, 9, 15, 16 days)
		V	4	III			90	4	Diet IV (36 days)
		VI	12	III			90	12	Diet V (36 days)
		VII	11	IV			90	10	Killed

the coat became fuzzy and soft like that of a much younger rat. Xerophthalmia occurred regularly on diet II.

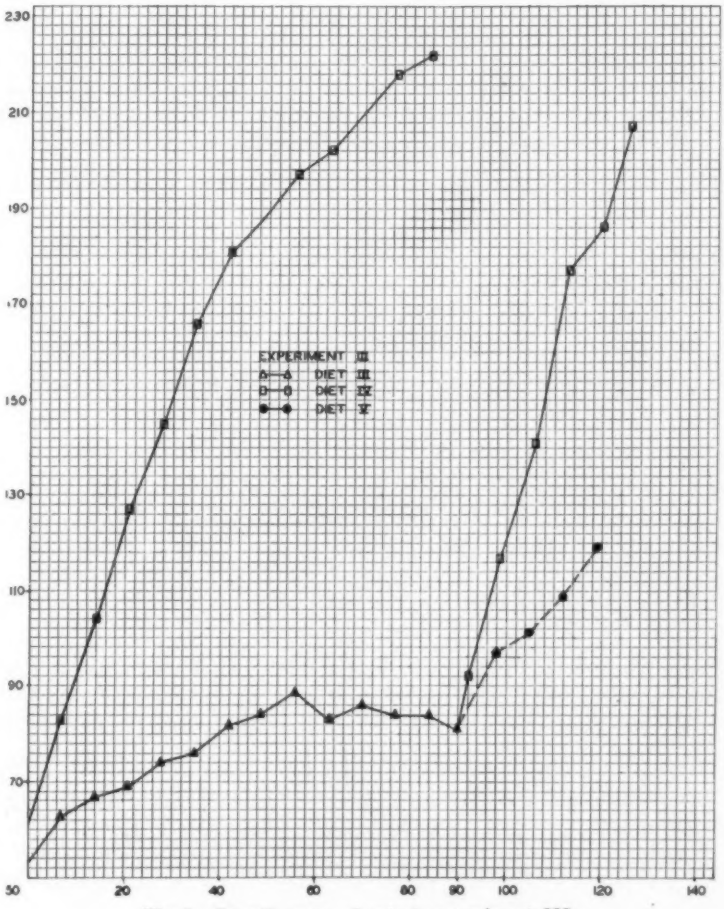


Fig. 1 Growth curve of rats in experiment III.

Other symptoms such as dermatitis of the digits described by György ('35) occurred occasionally. Contrary to the findings of Day, Langston and O'Brien ('31) cataract did not

occur except in one case and that only after autoclaved yeast had been added to the diet.

b. Microscopic. At 70 days there were only slight changes in the epithelium and sebaceous glands of the tail. After 90 days the epithelium becomes thinner and the amount of keratinized material much less. The growth of the basal cells appears to be greatly reduced, probably accounting for the decrease in keratin. The sebaceous glands in the earlier stages may show only a decrease in the amount of cytoplasm

Fig. 2 Gross appearance of tails. A. Photograph of tail of control rat on diet IV, experiment III. B. Photograph of tail of deficient rat after 100 days on diet III, experiment III.

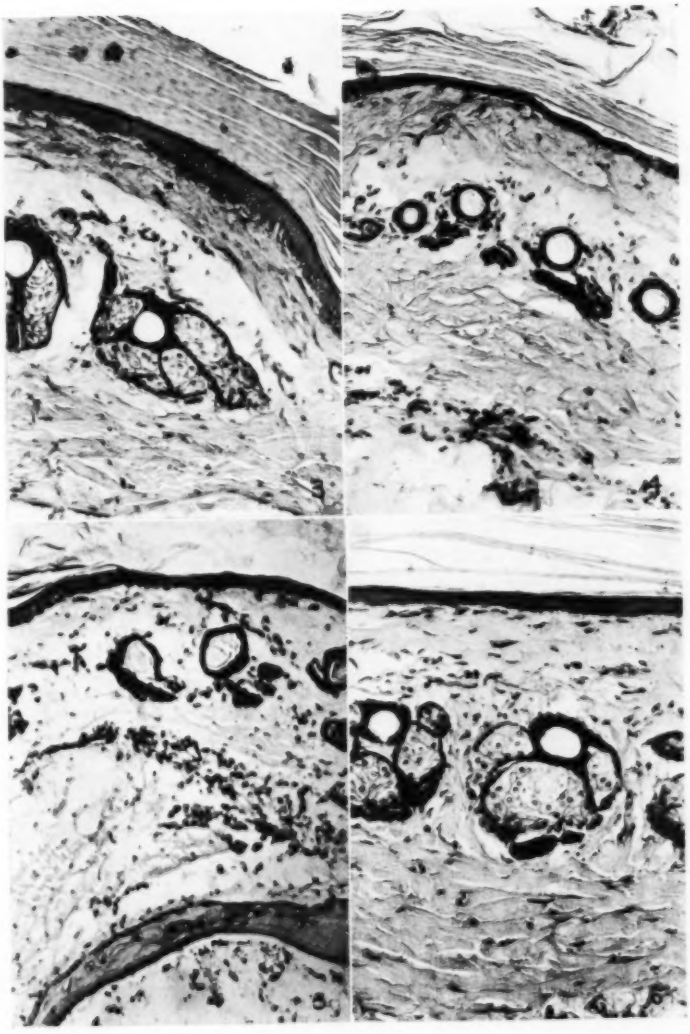
but later the number of cells decreases and in some of the rats killed after 100 days they are represented only by a few cells with very little cytoplasm. The fat which in the normal surrounds the glands is replaced by connective tissue having

Fig. 3 Photomicrograph of cross section of the tail of control rat on diet IV, experiment III. Note sebaceous glands, epithelium and keratin. × 150.

Fig. 4 Photomicrograph of cross section of the tail of a deficient rat on diet III for 100 days in experiment III. Note extensive atrophy of sebaceous glands and epithelium and also the decrease of keratin. × 150.

Fig. 5 Photomicrograph of cross section of the tail of a deficient rat on diet III for 90 days in experiment III. Note atrophy of sebaceous glands and epithelium. × 150.

Fig. 6 Photomicrograph of the cross section of the same rat as shown in figure 5, 36 days after autoclaved yeast had been added to the diet. Note the almost complete return to normal of the sebaceous glands and epithelium. × 150.



Figures 3 to 6

a peculiar hyalinized appearance. There is no round cell infiltration, accumulation of polymorphonuclear leucocyte or any other change which would indicate an infection.

Regeneration occurs when the diet is supplemented with autoclaved yeast (diet IV). This was not evident in two rats after 6 days on the supplemented diet, was definite but slight in one rat after 9 days, was more marked in two rats after 15 and 16 days, respectively, and apparently complete in four rats after 36 days.

Some regeneration occurred in rats given egg white (diet V) but this was less marked just as their growth rate was slower than in the case of rats receiving the autoclaved yeast.

Control experiments. In order to see whether the changes described above were due to inanition or were specific for vitamin G the following experiments were carried out.

1. Eight rats were placed on Steenbock and Black's ('25) rickets producing diet no. 2965 at the age of 28 days and maintained on it for 66 days when rickets could be demonstrated both clinically and by roentgenogram. Cross sections of the tails revealed practically normal sebaceous glands.

2. Ten rats were deprived of food but given water. This resulted in gradual loss of weight, general weakness and death, but no characteristic lesion. Microscopic preparations of the tails of these rats show a replacement of the fat around the sebaceous glands with connective tissue but no change in the epithelium or sebaceous glands themselves.

3. Twelve rats were given food but deprived of water. These also lost weight and died, but in addition all the rats developed the blood stained wrists often described as a symptom in vitamin G deficiency (Chick and Roscoe, '28; Kon, '31; Akroyd, '30; Sherman and Derbigny, '32). The mouth, nose and eyes were also stained. Microscopic preparations of the tails of these rats were essentially the same as those deprived of food.

4. Ten rats were placed on Sherman's A free diet (Sherman and Smith, '31) at the age of 28 days and maintained on it for 66 days. At this time they all showed xerophthalmia,

the weight was decreasing rapidly, one died on the sixty-sixth day and the others were obviously moribund. Abcesses were present at the base of the tongue at autopsy in all ten rats. Microscopic preparations of the tails revealed a marked atrophy of the epithelium, the sebaceous glands showed a slight decrease in the size of the cells and in some instances they were represented by only a few small cells. The fat around these glands had disappeared, its place being taken by young connective tissue.

5. Ten 28-day-old rats were placed on a B deficient diet identical with that of Chase and Sherman ('31) except that the vitamin G was supplied by brewers' yeast autoclaved 2½ hours at 15 pounds pressure instead of autoclaved baker's yeast. They were maintained on it until they died, all showing the characteristic neuromuscular changes before death. The microscopic tail sections showed essentially the same thing as those of the A-deficient rats with the exception that the sebaceous gland change was more and the epithelial change less marked.

DISCUSSION

We have presented evidence to show that an atrophy of the sebaceous glands and thinning of the epithelium together with hyalinization of the connective tissue occurs in the tails of rats subsisting on a diet deficient in the vitamin G complex. This does not occur in rats on the same diet supplemented with autoclaved yeast. After the atrophy has occurred in any vitamin G deficient rat, as demonstrated by biopsy section, there is prompt and complete regeneration upon the addition of autoclaved yeast to the diet. However, we cannot say that this characteristic change is specific for vitamin G as it also occurred to some extent in the rats on vitamin A deficiency and in the rats on vitamin B deficiency in our control experiments. There are three possible explanations for the pathological changes observed 1) they may be due to chronic inanition, 2) in the case of the vitamin A and vitamin B deficiencies there may be a secondary shortage of vitamin G

due to inanition or faulty absorption, 3) vitamins A, B and G may all be necessary for the protection of the tissues involved. Under these circumstances the pathological changes described may occur when any one of them is lacking. Experiments are now in progress to test these hypothetical explanations.

It should be emphasized that in the rats deprived of water, the blood stained wrists occurred regularly and became gradually worse until the rat died. This is often described as a symptom of vitamin G deficiency, but it also occurs in other deficiencies and frequently accompanies infections. It is nonspecific and apparently results from dehydration.

It is impossible to state what factor or combination of factors is responsible for maintaining the characteristic structure of the sebaceous glands and epithelium. The fact that abnormal sebaceous secretion has been reported in cases of human pellagra (Bigland, '20; Biggam and Ghalioungui, '33; and Corkill, '34) and the results of the present studies lend some support to the idea of vitamin G deficiency. Corkill made his observations on Sudanese millet eaters which would preclude any effect due merely to corn meal. He states that in pellagra the sebaceous glands hypertrophy. There was no histological evidence presented for this, however, and the subsequent observation that there is a 'yellowish secretion' which has been aptly likened to 'sulfur flakes' indicates an abnormal secretion. We attempted to determine by means of bi-weekly biopsies on the tails of rats whether or not an hypertrophy preceded the atrophy of the sebaceous glands. This experiment was inconclusive, however, since the bleeding and the infection obscured both the gross and microscopic results. We have observed engorged sebaceous glands palpable to the touch in pellagra patients in this hospital and have also seen the 'sulfur flake' type of secretion.

No attempt has been made to differentiate the factors of the vitamin G complex as described by the English investigators (György, '35 confirmed by Harris, '35 and Chick, Copping and Edgar, '35). The fact that we did not get the characteristic dermatitis of the digits regularly may be explained by the

possible contamination of either the cornstarch (Hogan and Richardson, '34) or the source of B with the protective factor.

SUMMARY AND CONCLUSIONS

1. Seventy-four rats given Bourquin and Sherman's G-deficient diet for 90 days or longer showed varying degrees of atrophy of the sebaceous glands and thinning of the epithelium in cross sections of the tail. The fat normally present around the sebaceous glands was replaced by connective tissue. There was no cellular infiltration.

2. The positive control rats, thirteen receiving the same G-deficient diet, supplemented with autoclaved yeast and nineteen receiving the stock diet had normal sebaceous glands and epithelium.

3. After the change in the sebaceous glands and epithelium was produced in sixteen rats as shown by biopsy, autoclaved yeast was added to the diet. In every case where the rat lived longer than 9 days there was evidence of regeneration and by the end of 36 days it was practically complete, the sebaceous glands and epithelium appearing normal.

4. No sebaceous gland or epithelial changes were noted in ten rats deprived of food nor in twelve rats deprived of water. The latter, however, had the blood stained wrists so often described as a symptom in vitamin G deficiency.

5. In the vitamin control experiments some changes in the epithelium and sebaceous glands were noted in vitamin A deficiency and in vitamin B deficiency. There was no appreciable change in the rats on Steenbock's rickets producing diet.

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HUMAN IODINE BALANCE

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NINE FIGURES

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In comparison with the work on other phases of iodine metabolism, there is a paucity of work on iodine balance in human subjects. The only long study which we have available on a single individual is that of von Fellenberg on himself ('26). He analyzed urine, stool, nasal secretion and sweat. His studies were carried out consecutively on four levels of intake: 17, 55, 75 and 80 micrograms of iodine per day. The basal diet was the same throughout. The iodine was added in various forms as indicated. Complete studies were made for 6 days on the 17 micrograms intake after he had been on the diet for 21 days. The average total excretion per day was 14.2 or 84 per cent of that ingested, the nasal and sweat secretion accounting for about 15 per cent. For 7 days immediately following this he increased his intake to 55 to 58 micrograms of iodine per day by adding KI. Complete analyses were reported for the last 6 days. The average daily iodine output was 38 micrograms per day or 67 per cent of that ingested. Ten per cent was accounted for in the sweat and nasal secretion. Twenty-four micrograms of this was in the urine. By replacing the KI with cod liver oil the iodine intake was raised to 75 micrograms per day for 6 days. The average daily iodine excretion was 37 micrograms per day or only 50 per cent of that ingested. The distribution of the excreted iodine was the same as that in the period preceding.

Immediately following this for 5 days sardines or watercress were added to the diet to make the iodine content 77.5 to 80 micrograms of iodine per day. The excretion of iodine through the nasal secretion and sweat remained unchanged. The urinary iodine varied from 21 to 58 and the total excretion from 36 to 97 with an average of 63 micrograms of iodine or 80 per cent of that ingested. Following this increased iodine feeding the intake was decreased at once to the original level and the results for 5 days reported. The average iodine output per day was 38 micrograms and the output was as great on the fifth day as on the first. From these data several facts seem evident. Sweat and nasal iodine excretion were uninfluenced by changes in the iodine intake, stool iodine was only slightly altered. The iodine in KI is excreted more rapidly than the iodine in cod liver oil, sardines or watercress. When the intake of iodine was suddenly decreased the subject was not approaching balance at the end of 5 days.

Scheffer ('33 a, '33 b) carried out balance studies on twelve normal subjects with balance periods of 3 or 5 days. He analyzed urine, stool and sweat. Intake levels studied were 54, 59, 66, 130 and 155. In two patients with intakes of 54 micrograms of iodine per day the total outputs were 53 and 49. Of this, 18 and 29 were in the sweat. Then in these two subjects 98 and 90 per cent of the intake was accounted for through excretion. The combined urine and stool output accounted for only 65 and 37 per cent. One subject excreted 28 in the urine, 3.1 in the stool, and 10 in the sweat. Only 53 per cent of the intake was accounted for in the urine and stool combined. The iodine excretion through urine and stool was reported on seven individuals whose intakes varied from 62 to 70 micrograms of iodine per day. The combined excretion in these individuals varied from 21 to 28 micrograms of iodine per day or an excretion of 30, 33, 34, 34, 37, 42 and 44 per cent of that ingested. One subject with an intake of 130 micrograms per day excreted 57 in the urine, 2.8 in the stool and 45 through the skin. The combined excretion was 105 or 81 per cent of that ingested. The urine and stool accounted for

46 per cent of that ingested. Another normal subject with a daily intake of 155 micrograms excreted 116 micrograms in the urine and 24 in the sweat giving a combined excretion of 140. Seventy-five per cent of that ingested was accounted for in the urine alone. The fecal iodine was mentioned in only a few instances because of its constancy regardless of intake. The fecal iodine varied from 2 to 10 micrograms per day with an average of less than 5 micrograms per day. The same was true of the fecal iodine in von Fellenberg's study.

Attention is called to fecal iodine at this time because it is exceedingly important in the hyperthyroid patients as shown by Scheffer's work ('33, '34). Scheffer carried out three studies on subjects with goiter without hyperthyroidism, seven on subjects with goiter and hyperthyroidism, and six on subjects with Basedow's disease. With goiter, with or without hyperthyroidism, the iodine output in the urine was decreased. In goiter alone, the iodine output through the lungs was increased. In hyperthyroidism, with or without goiter, the fecal iodine was increased to several times the normal value. In Basedow's disease the iodine output through the skin was also increases.

In the three cases of goiter without hyperthyroidism the results were as follows: with an intake of 80 micrograms of iodine per day, there was 15 in the urine, 5 in the stool, and 40 through the lungs, giving a total excretion of 60 micrograms per day or 75 per cent of the intake; with 105 ingested, 9 micrograms per day was excreted in the urine, 9.5 in the stool, and 15 through the lungs, total 33 micrograms of iodine per day or 31 per cent of that ingested; with an intake of 139, excretion in the urine was 14.6, in the stool, 4.7, and through the lungs, 30 micrograms of iodine per day, totaling 49 micrograms or 35 per cent of that ingested.

In hyperthyroidism with goiter, urine and stool iodine are reported on seven cases of known intake; sweat excretion on three, and lung excretion on one. Four patients with intakes of 80 to 100 micrograms per day excreted from 2.5 to 9.0 micrograms per day in the urine, while a fifth on the same

intake excreted 25. The five excreted from 33 to 68 micrograms per day in the stool. The excretion through the skin in the three cases studied was 2.1, 14 and 16 micrograms per day. The total excretion varied from 40 to 91 with an average of 70 micrograms per day. One patient with an intake of 100 to 120 eliminated 16 in the urine, 67 in the stool, 27 in the sweat, and 17 in the expired air. Total excretion was 127 micrograms per day. Another with an intake of 150 excreted 94 in the urine, 35 in the stool, and 20 in the sweat, making a total of 149.

In Basedow's disease with intakes varying from 75 to 110 the urinary excretion varied from 23 to 58 in four cases and was 265 in the fifth. Stool iodine varied from 24 to 61 in four cases and was 7 in another case. Iodine excretion through the skin in two cases was 216 and 109. Combined excretions varied from 119 to 289 with the exception of one case which excreted 69. The average of all the combined excretions was 184. One patient with an intake of 130 excreted 26 in the urine, 49 in the stool, and 53 in the sweat, giving a total of 128. These results will be discussed further in connection with our own.

PROCEDURE

Our investigations on iodine balance include analysis of intake, urine, stool and in some instances sweat. We used 3-day periods. We studied three subjects for five, one for three, and one for two consecutive periods. Our subjects included two normal persons, one patient with myxedema, and two patients with exophthalmic goiter. With the exception of subject E, no one had any iodine medication during the past 2 months.

The diet for each balance study was selected by the subject and remained unchanged throughout the experiment. The diets were calculated to contain 1 gm. of protein per kilo of body weight, and to have a caloric value of basal plus 20 to 30 per cent. The subjects were kept at bed rest and this regimen maintained them at constant weight.

An effort was made to secure as much uniformity as possible in the food used throughout the experiment. Canned goods for the entire study were of the same brand and bought at the same time. Meat and potatoes for the entire experiment were set aside at one time. Meat was ground, weighed, and frozen. Butter enough for the entire experiment was worked into one sample. Perishables were set aside for each period. Bread for 1 day was taken from a single loaf. The same brand was used throughout the experiments. Daily samples of the food except butter, sugar and salt, were weighed out and dried. Three-day samples were put together, ground, salt and sugar added, and aliquots taken for analysis. Butter and water were analyzed separately.

Daily urine samples were made up to 2 liters. Three-day samples were combined for analysis. Carmine was given to mark the feces 20 to 30 minutes before breakfast at the beginning of each period. The stools were divided, dried, and ground for analysis. When sweat was collected, the subjects wore loosely woven cotton underwear which had been washed in double distilled water. Towels which had been washed in double distilled water were used for bathing. Each day the underwear was changed. The subjects were bathed in 6 liters of water. The underwear and towels were washed through this water and then they were rinsed three times, making the total volume 10 liters. Aliquots from 3 days were combined for analysis. With C and D, tap water was used. For E, iodine-free water prepared by distillation from base was used.

The analyses on A and B were done by Phillips and Curtis' ('34) modification of the von Fellenberg method and those on C, D and E by McCullagh's method ('34).

The protocols of the subjects used in this experiment are given below:

Subject A, 336247, unemployed white male, 28 years of age entered the hospital 12/3/33 for this balance study. Physical and laboratory examinations were negative. Weight 120 pounds, B.M.R. minus 6, T. —98, P. —60, R. —12, B.P. 92/60. He was placed on the diet 12/4/33 and the first carmine given 12/6/33.

Subject B, 340597, white female shoe stitcher, 31 years of age, entered the hospital 2/3/34 for this iodine balance study. Weight 109 pounds, B.M.R. minus 10, T. —98.2, P. —69, R. —16, B.P. 110/70. The subject was placed on the diet 2/5/34, and the first carmine given 2/7/34.

Subject C, 335531, white housewife, 38 years of age, entered the hospital 10/27/33 with the signs and symptoms of essential hypertension. 11/31/33 thyroidectomy was performed leaving at the left a portion not larger than $10 \times 7 \times 3$ mm., and on the right a portion not larger than $10 \times 10 \times 2$ mm. B.M.R. before the operation was plus 3, and 2 weeks following operation it was minus 13. 4/8/34, 4½ months after thyroidectomy, the patient entered the hospital for a general check-up presenting a characteristic picture of myxedema. B.M.R. was minus 28, T. —97, P. —56, and the weight was 133, or 13 pounds higher than before operation. The subject was placed on the diet 4/10 and the collections started 4/12.

Subject D, 341454, white housewife, 24 years of age entered the hospital 3/27/34 with the following complaints of 5 months' duration: prominent eyes, nervousness, tremor, emotional and heat instability, tachycardia, dyspnea and hoarseness. Positive physical findings were a soft diffusely enlarged thyroid, pulse of 120, a soft systolic murmur best heard over the apex and transmitted over the entire precordium, blood pressure 148/84. 4/10/34 the patient was started on the experimental diet, and the first carmine was given 4/12. B.M.R. taken during the experiment was plus 28, T. —98, P. —100, R. —20, B.P. 110/46.

Subject E, 345595, white housewife, 24 years of age, entered the hospital 10/30/34 with the following complaints of 6 months' duration: nervousness, ease of fatigue, enlargement of neck, and tachycardia. The patient had rubbed a patent medicine on her neck for 2 months and stopped using it 2 weeks before entering the hospital. This medicine was found to contain 1.3 gm. of iodine per 100 cc. We did not obtain this part of the history until the study was nearly finished. The patient had denied medication on three different occasions when the history was being checked. Positive physical findings were a warm moist skin, enlarged thyroid, pounding heart with shock over the apex, pulse 100, and fine tremor. The B.M.R. was plus 65, T. —98.4, P. —98, R. —15, B.P. 120/60.

RESULTS AND DISCUSSION

Subject A ingested 156 micrograms of iodine per day; the observations are shown in figures 1 and 2 as micrograms of iodine per day. The results on A were as follows: Period I, in the urine 51, in the stool 38, combined 89, positive balance 67; period II, in the urine 44, in the stool 19, combined 63, positive balance 93; period III, in the urine 53, in the stool 30, combined 83, positive balance 73; period IV, in the urine 43, in the stool 31, combined 74, positive balance 82; period V, in the urine 47, in the stool 16, combined 63, positive balance 93. The average urinary iodine output for the 15 days studied was 47, fecal iodine 27, combined 74, and balance 82. Only 47 per cent of the intake was accounted for by the urine and feces. This is less iodine in the urine and stool than Scheffer found in a subject of similar intake but the same as one with slightly lower intake.

Subject B ingested 56 micrograms of iodine per day and the results are shown in figures 3 and 4. The results on B were as follows: Period I, in the urine 36, in the stool 8, combined 44, positive balance 12; period II, in the urine 36, in the stool 6, combined 42, positive balance 14; period III, in the urine 36, in the stool 9, combined 45, positive balance 11; period IV, in the urine 31, in the stool 10, combined 41, positive balance 15; period V, in the urine 35, in the stool 12, combined 47, positive balance 9. The average urinary iodine output was 35 micrograms per day, fecal 9, making a combined excretion of 44 micrograms of iodine per day or 75 per cent of the iodine intake. Von Fellenberg accounted for about the same percentage on himself when he ingested 17 micrograms of iodine per day and also 80 micrograms. One of Scheffers' subjects on a higher intake showed a similar percentage output. The other subjects tended to agree more nearly with the results on A.

Sweat loss may have been an important factor in the great difference between A and B. A had a consistently moist skin and was restless, while B had a dry skin and remained quietly in bed. The great importance of sweat had been emphasized

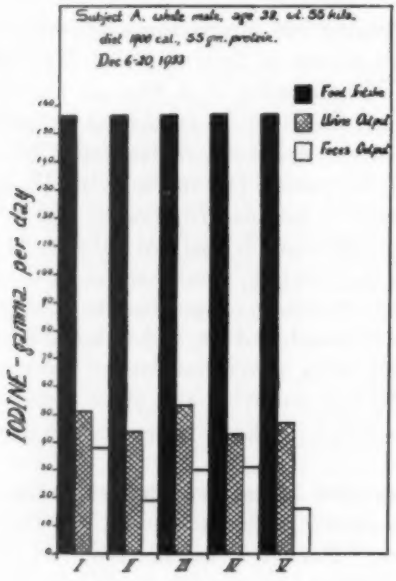

Figure 1

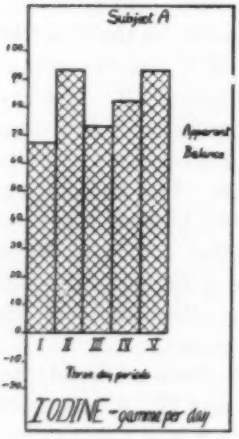

Figure 2

by Scheffer but we had hoped by bed rest in a room of 70 to 75°F. to render this factor constant. The effect of a previous diet, shown by von Fellenberg's work, may have been a con-

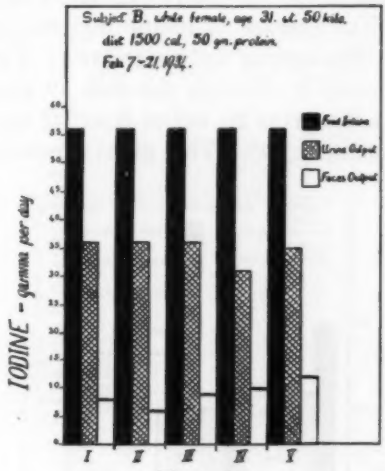

Figure 3

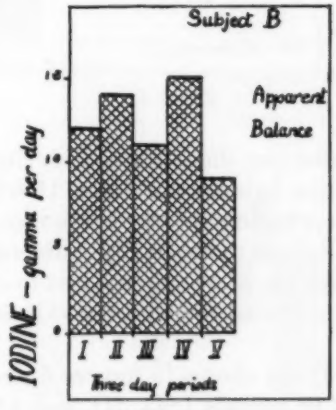

Figure 4

tributing factor here. However, the relative constancy on five consecutive periods leads us to believe that this is not very probable.

In the next three subjects, on which we made sweat determinations, balances are given as apparent balances without the sweat being subtracted, and as balance when the sweat is considered. The results on C are shown in figure 5. Two periods only were run. The amount of iodine ingested was 39 micrograms per day. The output was as follows: Period I, in the urine 24, in the stool 9, through the skin 19 micrograms per day; period II, in the urine 20, in the stool 10, and through the skin 20 micrograms per day. This gives a positive balance of

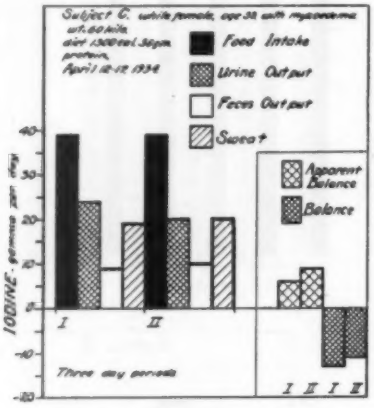

Figure 5

6 and 9 micrograms per day, not considering the sweat excretion or a negative balance of 13 and 11 when the sweat is considered. This experiment was not continued long enough to draw any conclusions unless many more such studies show similar results. We are at a complete loss to explain the great amount of iodine excreted through the skin in a case of true myxedema.

The results on D are shown in figures 6 and 7. The intake of iodine was 162 in periods I, II, III, and 147 in periods IV and V. Some vegetables were changed at the request of the patient in periods IV and V. The output was as follows: Period I, in the urine 46, in the stool 94, in the sweat 15, total

155, positive balance 7; period II, in the urine 63, in the stool 90, in the sweat 16, combined 169, negative balance 7; period III, in the urine 43, in the stool 99, in the sweat 13, total 155, positive balance 7; period IV, in the urine 64, in the stool 95,

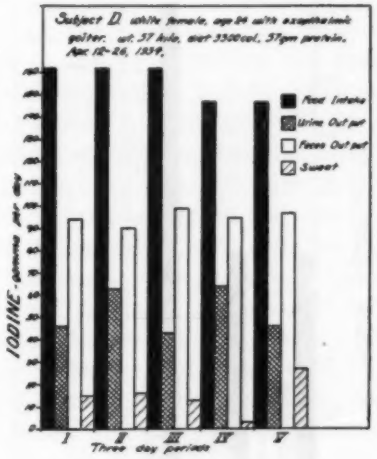

Figure 6

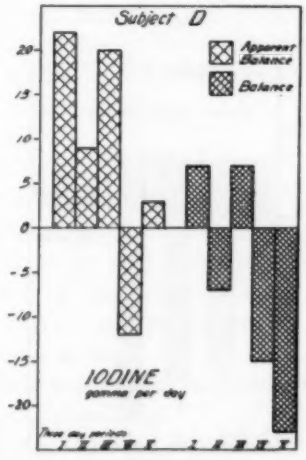

Figure 7

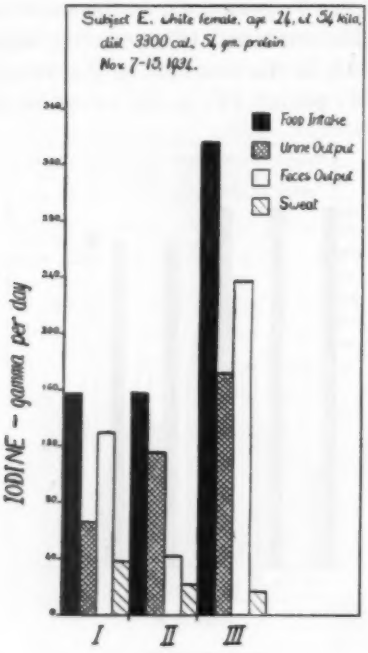

Figure 8

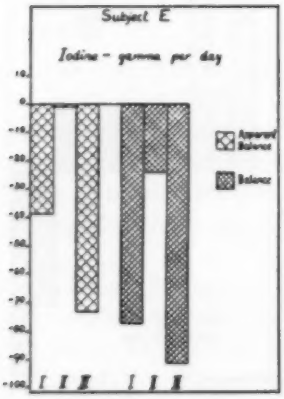

Figure 9

in the sweat 3, total 162, negative balance 15; period V in the urine 46, in the stool 97, in the sweat 27, combined 170, negative balance 24. It will be noticed that the striking thing here is the large output of iodine in the feces.

The results on subject E are shown in figures 8 and 9. The iodine ingested in the food was 158 micrograms per day throughout, but in the third period the iodine intake was raised to 336 with potassium iodide. The iodine excretion was as follows: Period I, in the urine 67, in the stool 130, in the sweat 38, combined 235, negative balance 77; period II, in the urine 116, in the stool 43, in the sweat 23, combined 182, negative balance 24; period III, in the urine 172, in the stool 237, in the sweat 18, combined 427, negative balance 91. The large negative balance here is perhaps a result of the iodine medication received 2 weeks previously. We may assume, however, that since the fecal iodine is little influenced by small changes in intake previous medication would have little effect on it. Therefore this would appear to be further evidence for Scheffer's contention that there is an increased fecal loss of iodine in hyperthyroidism. The reason for our not confirming the very large sweat loss in hyperthyroidism found by Scheffer is doubtless due to the fact that our patients were kept comfortable because it was easier to handle them that way. This meant that their rooms were a little cooler and they had less bedding than the other subjects.

SUMMARY

1. Iodine balance was studied in 3-day periods for 15 days on three subjects, nine on a fourth, and six on a fifth.

2. The combined urine and stool iodine may account for less than 50 per cent of the iodine intake.

3. There was a fairly uniform output of iodine for an individual on a monotonous diet.

4. The fecal iodine was higher in these studies than in those of Scheffer and von Fellenberg.

5. In two subjects studied Scheffer's observation of an increased fecal iodine in hyperthyroidism was confirmed.

ACKNOWLEDGMENT

The authors wish to acknowledge the invaluable cooperation of Mary L. Bone, dietician of the Starling-Loving Hospital, and Ione Sundstrom, head nurse of the Research Division of the hospital.

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THE UTILIZATION OF INULIN FOR GROWTH BY THE YOUNG WHITE RAT

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ONE FIGURE

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Despite much experimental study, the value of inulin for the nutrition of the higher forms is yet to be demonstrated satisfactorily. It is recognized that enzymes, which convert inulin to fructose, can hardly be significant factors in the activity of the secretions of the gastro-intestinal canal of the higher forms, but that under certain conditions, the gastric acidity may be sufficiently high to cause the hydrolysis of inulin to fructose to a limited extent. The microflora of the intestine attack inulin readily, but the chief products of this fermentation are fatty acids of low molecular weight and not carbohydrates (Ashe and Bing, '34). Absorption and utilization of inulin without previous hydrolysis in the gastro-intestinal tract has also been suggested (Heupke and Blanckenburg, '34), but in the light of experiments on the fate of inulin introduced parenterally, this seems improbable. McCance and Lawrence ('29) have presented an excellent critical review of the general problem of inulin utilization.

The chief evidence in support of utilization of inulin is to be obtained from certain experiments in which a deposition of liver glycogen has been observed to follow inulin feeding and from increases in respiratory quotients after the administration of inulin. In most cases, the increases in liver glycogen after ingestion of inulin are slight, particularly

when compared with the values obtained after the feeding of comparable amounts of readily utilized carbohydrates, fructose or glucose. The fallacies of the evidence based on the respiratory quotient are well presented by McCance and Lawrence ('29).

In addition to lack of satisfactory direct experimental methods and to difficulties in the interpretation of data, still further confusion has resulted from the failure to recognize the variability of the material used as a source of the carbohydrate. It is known that the chemical and physical properties of inulins of different botanical origin vary (Yanovsky and Kingsbury, '31, '33), and that fructosans, other than inulin, are present in plant tissue. Moreover the relative amounts of inulin and the other fructosans, the so-called inulides, vary with the season. It can hardly be expected, therefore, that the results obtained by the administration of a tuber (e.g., Jerusalem artichoke), which contains inulin and other fructosans, will be comparable with those obtained with inulin isolated from the same source. In fact inulins of different origin, although prepared under uniform conditions, may not be chemical individuals, and it is preferable in the light of our present knowledge to include in the inulin group all complex anhydrides of fructose (Yanovsky and Kingsbury, '31).

One direct method of study, which is concerned with the calorific value of inulin in the diet of the young white rat, has not been employed extensively. If inulin is used to any significant extent, it should be able to supply energy and thus promote the growth of young white rats when supplied either as the sole source of carbohydrate, or as a supplement to readily available carbohydrates in a low calorie diet adequate in all other known essential elements. The present study employs this procedure and approaches the inulin problem by a consideration of the rate of growth of young white rats maintained on a low calorie diet with sucrose or fructose as the source of carbohydrate in the basal diet and inulin or inulin plus sucrose or fructose in the experimental diets.

Purified inulin from chicory, generously furnished through the courtesy of Dr. Elias Yanovsky of the carbohydrate division of the Bureau of Chemistry and Soils of the United States Department of Agriculture, was used in our studies.

The results have indicated some utilization of inulin as a source of energy for the growing white rat, a utilization, however, distinctly inferior to that of either sucrose or fructose. In common with other observers, we have noted that inulin, if ingested in considerable amounts, resulted in marked gas formation and disturbance of function of the gastro-intestinal canal. We feel that our data indicate that the isolated polysaccharide from chicory is of little dietetic value from the practical standpoint as a significant source of energy. Our results are at variance with those of certain Japanese investigators, which will be considered subsequently.

EXPERIMENTAL

The chicory inulin used in these studies contained 10 per cent of moisture and 0.09 per cent of ash and showed a specific rotation of $-33.9°$ (uncorrected for moisture or ash) when examined at 27° with sodium light.

Litter units of young white rats of 65 to 75 gm. weight were divided into control and experimental groups for the nutritional studies to be described. Attempts to use younger and smaller rats were unsuccessful since, even when relatively small amounts of inulin (1.5 gm.) were added as supplements to the basal diet, marked diarrhoea and distention of the intestines resulted and the animals died within a few days.

In preliminary experiments it was found that inulin was not satisfactory as the sole source of carbohydrate of the diet, that some readily utilizable carbohydrate must be supplied. The addition of 2.5 to 4.1 gm. of inulin to a basal diet containing no readily available sugar resulted in a rapid loss of weight and death within a few days. Death was presumably due to starvation and the marked distention of the intestines occasioned by the large amount of gas produced as a result of the fermentation of the inulin. The distention of

the abdomen was so marked that the animals could be readily distinguished from the controls. Diarrhoea and frequently bloody fecal masses were observed.

In the final series of experiments, basal low calorie diets containing a limited amount of sucrose (diet 10) or fructose (diet 12) were prepared to which extra carbohydrate in the form of sucrose, fructose or inulin was added for the experimental diets. The composition of these diets is presented in table 1.

TABLE 1

Basal and experimental diets

	10	10a	10c	11	12	12a	12c
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Casein	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Cod liver oil	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lard	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Agar	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt mixture[1]	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sucrose	1.5	1.5	3.0				
Inulin		1.5		1.5		1.5	
Fructose					1.5	1.5	3.0
Daily food intake	3.8	5.3	5.3	3.8	3.8	5.3	5.3
Daily calorie intake (calculated)	12.6	18.6[2]	18.6	12.6[2]	12.6	18.6[2]	18.6

[1] McCollum-Davis salt mixture no. 185.

[2] Calculated on the basis that 1 gm. of inulin supplies the same number of calories as 1 gm. sucrose or fructose.

In each series of experiments, the rats were placed in individual wire cages and given a fixed amount of food daily, 3.8 gm. for each of the animals receiving diets 10, 11 and 12, and 5.3 gm. for each of those receiving diets 10a, 12a, 12c and 10c. A supplement of 50 mg. of Harris yeast vitamin pill was given daily to each rat to supply the vitamin B complex. In two series of experiments a daily supplement of 100 mg. of the vitamin pill was given daily to each rat to see whether a larger amount of vitamin B would influence the growth of the rats. Water was given ad libitum. The rats were weighed every 3 days. Consumption of food was both rapid and complete. This was to be expected as the amount of food given daily to each rat was quite small (3.8 or 5.3 gm.).

Typical results obtained with various litter units are presented in table 2 and figure 1. The diets fed were essentially the same except for the content of carbohydrates. The basal diet, which furnished approximately 12.6 Cal., was calculated to be sufficient to maintain the animals at nearly constant

TABLE 2

Weight of rats fed low calorie diets and diets containing added carbohydrate. All feeding periods were of 60 days duration

SEX	DIET	WEIGHT		CHANGE IN WEIGHT	SEX	DIET	WEIGHT		CHANGE IN WEIGHT
		Initial	Final				Initial	Final	
Litter A					Litter B				
		gm.	gm.	gm.			gm.	gm.	gm.
F	10	60.6	65.5	+ 4.9	F	12	70.0	75.0	+ 5.0
F	10	71.5	81.5	+ 10.0	M	12	70.2	75.5	+ 5.3
M	10	65.0	80.0	+ 14.5	M	12	64.5	81.0	+ 16.5
F	11	69.0	62.5	— 6.5	F	12	64.5	78.5	+ 14.0
M	11	76.0	62.0	— 14.0	M	12c	77.5	113.5	+ 36.0
F	11	76.0	57.5	— 18.5	F	12c	62.5	102.5	+ 40.0
F	10a	70.5	102.0	+ 31.5	F	12c	71.5	112.0	+ 40.5
M	10a	64.5	105.0	+ 40.5	M	12c	70.5	119.0	+ 49.0
F	10a	67.0	99.0	+ 32.0	F	12a	70.1	99.0	+ 28.9
F	10a	63.1	104.5	+ 41.4	F	12a	67.5	99.5	+ 32.0
					M	12a	67.0	94.5	+ 27.0
Litter C					Litter D				
F	10	71.0	74.0	+ 3.0	M	12	60.8	67.5	+ 6.7
F	10	63.5	68.0	+ 4.5	F	12	65.2	68.5	+ 3.3
M	10	68.5	79.5	+ 11.5	F	12	59.0	68.5	+ 9.5
F	10a	77.0	98.5	+ 21.5	M	12	52.0	60.5	+ 8.5
F	10a	71.0	86.5	+ 15.5	F	12a	61.0	81.5	+ 20.5
M	10c	82.5	119.8	+ 37.3	M	12a	69.0	87.0	+ 18.0
F	10c	72.0	113.5	+ 41.5	M	12	73.2	87.5	+ 14.3
F	10c	74.2	107.3	+ 33.1	F	12	58.5	79.5	+ 21.0

weight. If the inulin were utilized to any significant extent, addition of 1.5 gm. of inulin daily (6 Cal.) should result in increased growth, since it was considered that the protein of the diet was adequate for animals of this weight. In the first series of experiments, in which sucrose served as the source of carbohydrate in the basal diet (diet 10), added inulin

(diet 10a) produced definite increases in weight (table 2, litters A and C), indicating utilization of the polysaccharide. Since inulin is a fructosan, it was considered desirable to supply fructose rather than sucrose as the source of carbohydrate in the basal diet (diet 12). Addition of inulin to this diet (diet 12a) also resulted in an increased rate of growth (table 2, litters B and D). It appeared that inulin was available as a source of energy to some extent.

In order to determine how the utilization of inulin compared with that of sucrose or fructose under our experimental

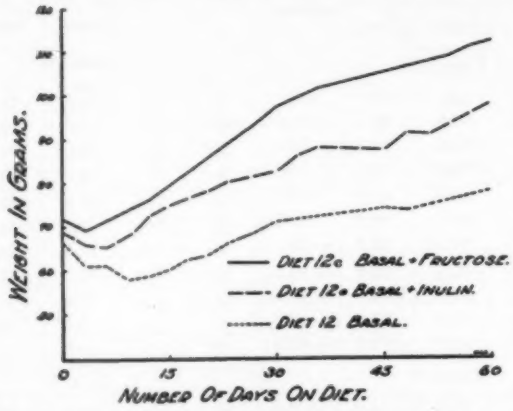

Fig. 1 Weight curves of rats receiving the basal low calorie diet and the basal diet supplemented by inulin and by fructose. The curves presented are averaged for three (diet 12a) and four (diets 12 and 12c) rats of the same litter.

conditions, sucrose (diet 10a) and fructose (diet 12c) were added to the basal diet in amounts comparable to the amount of the inulin added. It was observed that while, as already stated, rats fed the basal diets plus 1.5 gm. of inulin showed better growth than the control animals receiving the basal diet without supplement, the animals receiving the basal diet plus sucrose (litter C) or fructose (litter B) showed rates of growth distinctly superior to that of the inulin-fed rats. The figures show that although inulin may be utilized by the rat, the extent of utilization as measured by the growth response

CORRECTION

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Page 513, line 18. Sentence beginning "When half of the inulin of diet 11" should read "When the amount of inulin present in diet 11 was added as a supplement to the basal sucrose-containing diet (diet 10 a) all the animals grew well, an average gain per rat of 36.3 gm. being recorded."

is by no means comparable to that of either sucrose or fructose. The same conclusion is to be drawn from the typical growth curve presented in figure 1.

It has been stated previously that it was found necessary to include some readily utilizable carbohydrate in the diet and that when inulin was the sole source of carbohydrate, marked gastro-intestinal disturbances which terminated fatally within a few days resulted. With one litter only were we successful in continuing the use of the diet in which inulin was the sole source of the carbohydrate (diet 11) throughout the entire feeding period of 60 days. The results of this experiment are presented in table 2 (litter A). On the basal low calorie diet containing a limited amount of sucrose (diet 10), three animals gained an average of 9.8 gm. each in the 60-day period. When inulin served as the sole source of carbohydrate (diet 11), all the animals survived but lost weight at first rapidly, then more slowly (an average loss of weight of 13.0 gm.). When half of the inulin of diet 11 was replaced by sucrose (diet 10a), all the animals grew well, an average gain per rat of 36.3 gm. being recorded. The results with this litter are discussed in detail since these particular animals appeared to tolerate inulin unusually well and the group fed both sucrose and inulin showed growth superior to that obtained on inulin feeding with any other litter. These results show clearly that inulin alone as a source of carbohydrate is unsatisfactory, but that inulin as a supplement to a diet containing some other carbohydrate has a limited value.

Our results which indicate that the utilization of inulin as a source of energy by young white rats is limited and is distinctly inferior to that of sucrose or fructose are not in accord with the results of a few similar studies in the literature. Emerique ('25) in studies similar to those here reported was unable to observe any utilization of inulin by young white mice. One group of only three animals was studied however. Ariyama and Takahasi ('29) believed that the nutritive value of inulin was equal to that of starch, dextrin, sucrose or lactose. The small number of rats (two) used makes the

experimental results difficult to interpret. Narushima ('27) found that inulin was as effectively utilized by young white rats as was cornstarch, but was not as effectively utilized as fructose. It seems incredible that the addition of 0.8, 2.4 and 2.8 Cal. as fructose (i.e., 0.2, 0.6 and 0.7 gm.) to a basal diet furnishing 37 Cal. daily could lead to such marked increases in weight (37.5, 37.5 and 34.5 gm., respectively), while the animals on the basal diet gained only 8.5 gm. during the same experimental period of 33 days. These results are the more striking since these small variations in the calorific value of the diet markedly influenced the growth of animals weighing for the most part over 100 gm. at the beginning of the feeding periods. Our results are rather in harmony with previous work from this laboratory (Bodey, Lewis and Huber, '27) in which it was shown that the administration of inulin led to an increased storage of glycogen in fasting young white rats but that the storage of glycogen was much less marked than that observed when fructose was fed.

In conclusion we wish to emphasize that, while our evidence indicates that the organism of the young rat may utilize inulin as a source of energy to a limited degree, the experiments furnish no information as to how this energy is made available. The lack of a known effective mechanism for the hydrolysis of inulin to fructose in the gastro-intestinal tract of the higher forms and the marked intestinal fermentation observed suggest that the nutritive value of the inulin may be due to the absorption and utilization of organic acid or similar products formed in the intestine by the activities of microorganisms (Ashe and Bing, '34). In addition, it should be pointed out that the results of the investigation do not bear directly on the problem of the utilization of inulin-containing vegetables. Our investigations are concerned with the isolated polysaccharide, inulin, from a single botanical source, chicory,[1] and the results cannot be applied to the problem of

[1] We also prepared in this laboratory inulin from freshly dug dahlia tubers. A limited series of studies with dahlia inulin gave results comparable with those obtained with inulin from chicory.

the utilization of the mixture of fructosans, the inulides, present in the vegetables, the nature of which varies with the season and with the botanical species.

SUMMARY

1. Young white rats (65 to 75 gm.) were maintained upon a diet, whose limiting factor was a calorific one.

2. When extra calories in the form of inulin from chicory, sucrose, or fructose were supplied as a supplement to the basal diet, the rate of growth was increased. The utilization of inulin under these experimental conditions, as measured by the response in growth, was distinctly inferior to that of sucrose or fructose.

3. Inulin could not be effectively utilized by the young white rat as the sole source of carbohydrate since the marked intestinal fermentation of the polysaccharide caused distention of the intestine and usually death within a few days.

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THE EFFECT OF CEREAL DIETS ON THE COMPOSITION OF THE BODY FAT OF THE RAT [1]

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Numerous studies have shown that the character of the body fat is materially modified by the diet. In feeding experiments with rats, Anderson and Mendel ('28) reported that with some dietary oils—soybean, corn, cottonseed and peanut—a close parallelism existed between the iodine number of the body fat and the iodine number of the food fat when the latter furnished 60 per cent of the total energy intake. Body fat synthesized from either carbohydrate or protein, so-called physiological fat, exhibited an iodine number of about 63. The present communication relates to a study of the character of fat produced when rations rich in cereals, which contain large amounts of carbohydrate and comparatively small amounts of fat, are fed. Do the individual cereal grains, corn, oats, wheat and barley, with an oil content ranging from 1.7 per cent to 5.3 per cent yield body fats of unlike composition? Inasmuch as cereal grains furnish the principal energy foods of human beings as well as of animals on which man depends for meat, milk, eggs, leather and wool, studies regarding any aspects of their nutritive properties ought to prove of interest and value.

[1] Presented before the Division of Biological Chemistry, Eighty-fourth meeting of the American Chemical Society, Denver, August 23, 1932.

[2] National research council fellow in medicine (1931–1932).

EXPERIMENTAL

Yellow corn, oat groats, wheat and hulled barley were used in the experiments. The analyses of these grains and of dried skimmed milk are recorded in table 1. With the exception of the determination of the oil content of each grain, analyses of the grains and of dried skimmed milk were made according to conventional methods. The amount of each cereal oil was determined by continuous extraction with hot alcohol; the extract was evaporated in vacuo, and the residue extracted with ether; the ethereal solution was dried with anhydrous sodium sulfate, filtered, and evaporated. The oil thus obtained was dried to constant weight. Diets containing

TABLE 1

Analyses of cereals and dried skim milk

CEREAL	MOISTURE	ASH	PROTEIN [1]	FAT	CARBOHYDRATE [2]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Yellow corn	8.5	1.3	8.7	4.0	77.5
Oat groats	6.9	2.0	16.1	5.3	69.7
Wheat (mixed)	8.5	1.7	11.1	1.7	77.0
Hulled barley	9.1	1.6	9.9	2.0	77.4
Dried skim milk	4.0	8.2	36.3	0.8	50.7

[1] Cereals, $N \times 5.7$; dried skim milk, $N \times 6.38$.

[2] By difference.

80 per cent finely ground cereal, 18.5 per cent dried skim milk, and 1.5 per cent salt mixture, were fed. The salt mixture was devised to supplement the mineral elements of the other dietary constituents. The composition of the diets and the relationship of the energy value of each cereal oil to the total energy value of the respective diet are recorded in table 2.

Each diet, supplemented once weekly with a cod liver oil concentrate rich in vitamins A and D, was fed ad libitum to a group of four or five female albino rats, which at weaning (21 days of age), and prior to initiation of the feeding experiments, were fasted for 48 hours in order to deplete the fat depots. The individual body weights at weaning ranged

from 35 to 45 gm. The feeding tests were conducted until the animals attained between approximately 190 and 220 gm. body weight.

The growth rate of the animals on the oat and corn diets was slightly superior to that shown by the normal growth curves for female rats (Smith and Bing, '28). Regarding the wheat and barley fed groups, the rate of growth of the former group approximated the normal, whereas the animals raised on the barley ration grew below the normal rate; the

TABLE 2

Composition of diets

DIETARY COMPONENT	OIL IN CEREAL	ENERGY VALUE OF CEREAL OIL	WEIGHT OF DIETARY COMPONENT	ENERGY VALUE OF DIETARY COMPONENT
	per cent	*per cent of total calories*	*per cent*	*per cent of total calories* [1]
Cereal				
Corn	4.0	8.0	80.0	82.0
Oats	5.3	10.0	80.0	82.0
Wheat	1.7	3.0	80.0	82.0
Barley	2.0	4.0	80.0	82.0
Dried skim milk			18.5	18.0
Salt mixture [2]			1.5	

A dose of 20 mg. cod liver oil concentrate[3] was administered once weekly to each rat

[1] Approximate values. The values for four cereals ranged from 81.7 to 82.9 cal.

[2] The salt mixture consisted of calcium carbonate 55 per cent, sodium chloride 40 per cent, ferric citrate 4.5 per cent and potassium iodide 0.5 per cent.

[3] The concentrate was kindly furnished by the Health Products Corporation, Newark, N. J.

individuals of the last-mentioned group weighed 20 to 30 gm. less at 125 days of age than those of the other groups.

Each rat was killed with illuminating gas, the gastro-intestinal tract removed, and the remaining tissue comminuted in a meat grinder. The lipids from this hashed tissue were obtained in most cases by a steam-rendering process described by Anderson and Mendel ('28). The lipids from the hashed tissue of one individual from each group were obtained by Bloor's ('26) hot alcohol-ether extraction method

and after the removal of acetone-insoluble substances (phospholipids), were compared in iodine value (table 3) with those obtained by the steam-rendering process which contained no acetone-insoluble substances. The iodine values were determined in duplicate by the Rosenmund and Kuhnhenn method

TABLE 3

Iodine number of body fats and of cereal oils

TYPE OF DIET	FINAL BODY WEIGHT OF RATS	IODINE NUMBER OF BODY FATS [1]	IODINE NUMBER OF CEREAL OIL [1]
	gm.		
Corn	219	81	116
	220	83	
	220	83	
	220	83	
	219	86[3]	
		Average 83	
Oats	220	76	99
	220	78	
	220	79	
	220	82[3]	
	220	82	
		Average 79	
Wheat	219	66	110
	176	70[3]	
	220	71	
	219	72	
		Average 70	
Barley	198	68	101
	188	69	
	188	71[3]	
	195	72	
	191	73	
		Average 71	
'Fat-free'		68[2] (Eckstein, '29)	
		64–71[2] (McAmis, Anderson and Mendel, '28–'29).	

[1] Rosenmund-Kuhnhenn method.
[2] Extracted with hot alcohol.
[3] Hanus method.

as modified by Yasuda ('31). In order to obtain further knowledge in regard to the make-up of the body fat, a sufficiently large sample, obtained by pooling all individual samples of each experimental group, was separated into its component saturated and unsaturated fatty acids by the lead-salt-ether method as described by Jamieson ('32). The data obtained are recorded in table 4.

TABLE 4

Distribution of fatty acids in body fat of rats[1]

TYPE OF DIET	IODINE NUMBER OF BODY FAT	TOTAL FATTY ACIDS RECOVERED	SATURATED FATTY ACIDS	UNSATURATED FATTY ACIDS	IODINE NUMBER OF UNSATURATED FATTY ACIDS
		per cent	*per cent*[2]	*per cent*[2]	
Corn	83	90.4	24.6	75.4	114
		90.0	25.3	74.7	116
Oats	79	93.1	28.5	71.5	114
		91.0	29.3	70.7	114
Wheat	70	93.3	30.0	70.0	103
		91.8	30.5	69.5	105
Barley	71	92.9	31.6	68.4	108
		93.2	31.2	68.8	108

[1] The average of two iodine number determinations of body fat is recorded; other figures represent the result of an individual analysis.

[2] Corrected percentage. The iodine numbers of the saturated acid fractions ranged from 6 to 11.

DISCUSSION

The average iodine numbers of 70 and 71 for the body fat, respectively, produced by rats fed the wheat and barley diets indicate that these animals deposited fat resembling that produced on 'fat-free' diets (Eckstein, '29; McAmis, Anderson and Mendel, '28–'29), whereas those fed corn and oats deposited fat definitely more unsaturated (table 3). Despite the fact that the oil of oats was less unsaturated (I.N. 99) than any of the other cereal oils, the comparatievly large oil content (5.3 per cent) is presumably responsible for the unsaturation (I.N. 79) of the body fat of rats fed the oats ration. Corn

oil was more unsaturated (I.N. 116) than any of the other oils, and the body fat of the rats fed the diet containing corn was correspondingly more unsaturated (I.N. 83) than the body fats of the other groups.

A study of the fatty acid composition of the various types of body fat indicated that the differences in iodine numbers were dependent both on the amount and character of the unsaturated fatty acids present (table 4). The body fat of the rats fed the corn diet contained the highest percentage of unsaturated acids and this fraction was the most unsaturated (I.N. 115) obtained. The body fat of the other three groups contained approximately the same percentage of unsaturated acids but differed in the iodine numbers of this fraction. The results suggest that some of the food unsaturated fatty acids are stored in preference to 'synthetic' fat when the food fat exceeds a certain minimal level, which level might be dependent upon the degree of unsaturation of the dietary fat.

SUMMARY

The cereal grains, corn, oats, wheat and barley, each of which has a low oil content, were included in individual diets of rats to furnish 82 per cent of the total energy value of the ration. The oil of corn and oats, constituting 4.0 per cent and 5.3 per cent, respectively, of the grains, supplied 8.0 and 10.0 per cent of the total calories of the corn and oats rations, respectively. Body fat produced by corn- and oats-fed rats, differed slightly in degree of unsaturation as measured by the iodine number (83 and 79, respectively). The oil of wheat and barley constituting 1.7 per cent and 2.0 per cent of the respective grains, supplied only 3.0 and 4.0 per cent of the total energy value of the respective wheat and barley diets. Body fat yielded by animals fed wheat and barley rations was less unsaturated (I.N. 70 and 71, respectively) than fat produced on either corn or oats diets, and was similar in character to fat obtained from rats fed 'fat-free' diets. As a result of a study of the total saturated and total unsaturated fatty acids of body fat obtained from rats on each cereal

ration, the unsaturated fatty acid content was found to range from about 68.5 per cent for fat from those fed the barley diet to 75.0 per cent for fat of rats on the corn ration.

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THE INFLUENCE OF DIET ON THE GLUCOSE TOLERANCE OF THE DOG

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THREE FIGURES

(Received for publication June 28, 1935)

The preceding diet may influence the glucose tolerance markedly. This question has been studied by Greenwald ('24), du Vigneaud ('25), Malmros ('28), Tolstoi ('29), Heinbecker ('28), Sweeny ('27 and '28), and Himsworth ('33). The procedure varied with respect to the diet, the duration of the experimental feeding, and the subject used. These variations probably account for the differences observed and for the differences in opinion held by the various investigators.

METHOD

The problem was to compare the effects of the different dietary regimes on the carbohydrate tolerance of each of six dogs. Three male and three non-pregnant female adult dogs were used. One male and one female dog of the original group become ill, and were replaced by two other dogs. The weight range was from 13 to 20 kg. The animals were mongrels, chosen from the supply of stock animals available at the time. The dogs were vermifuged before the experiment began. Each dog was kept in a large individual cage, and was allowed 6 hours of freedom daily in favorable weather, except on the days when glucose tolerance tests were performed.

The dogs were fed weighed portions of food twice each day, receiving one-half of their daily allowance at 9.00 A.M. and the other half at 3.00 P.M. Fresh water was available to each at all times except during the course of a glucose tolerance test. A fasting period of 16 hours preceded the glucose tolerance tests.

In selecting the diets, certain common foodstuffs were chosen, which could be combined in various ratios, to give the diets suited to the experimental needs. The composition of all the foods used in the experiment was taken from the values published by Atwater and Bryant ('06). The requirements to be satisfied were that the dogs would eat all the food given them, and neither gain nor lose weight to any extent. A value of 32 Cal. per kilogram per 24 hours was chosen as approximately the basal requirement for caged dogs, based on average figures reported by Steinhaus ('28). Each dog was given twice his basal requirement, or 64 Cal. per kilogram per 24 hours. This figure agrees fairly well with that of 70 Cal. per kilogram per day used by Morgulis and Edwards ('24) as a maintenance allowance for dogs under laboratory conditions.

The compositions of the various diets are shown below.

Balanced diet. Consisted of oatmeal and cornmeal (boiled together), boiled potatoes, lean steak, ground bacon, butter, and sodium chloride; 12.1 per cent protein, 27.8 per cent fat, and 60.1 per cent carbohydrate.

High protein no. 1. Consisted of granular gelatin, lean steak, cornmeal, ground bacon, and sodium chloride; 49 per cent protein, 19.93 per cent fat, and 31.07 per cent carbohydrate.

High fat no. 1. Consisted of boiled potatoes, lean steak, ground bacon, butter, and sodium chloride; 12.25 per cent protein, 75.24 per cent fat, and 12.51 per cent carbohydrate.

High carbohydrate. Consisted of boiled potatoes, cornmeal, honey, and sodium chloride; 8.82 per cent protein, 3.41 per cent fat, and 87.77 per cent carbohydrate.

High protein no. 2. Consisted of boiled egg, lean steak, cornmeal, bacon, and sodium chloride; 51 per cent protein, 22.6 per cent fat, and 26.4 per cent carbohydrate.

High protein no. 3. Consisted of boiled egg, lean steak, cornmeal, and sodium chloride; 74.4 per cent protein, 19.7 per cent fat, and 5.9 per cent carbohydrate.

High protein no. 4. Consisted of boiled egg, lean steak, cornmeal, bacon, and sodium chloride; 24.2 per cent protein, and 21.0 per cent fat, and 54.8 per cent carbohydrate.

High fat no. 2. Consisted of boiled egg, cornmeal, lean steak, bacon, butter, and sodium chloride; 15.9 per cent protein, 55 per cent fat, and 29.1 per cent carbohydrate.

High fat no. 3. Consisted of boiled egg, cornmeal, lean steak, bacon, butter, and sodium chloride; 13.8 per cent protein, 80.1 per cent fat, and 6.1 per cent carbohydrate.

In the first experimental period, the following scheme was used:

7 days on balanced; preliminary; dogs A to F, inclusive
7 days on high protein no. 1 (using gelatin), ended 7/27/34
7 days on balanced; ended 8/3/34
7 days on high fat no. 1; ended 8/10/34
7 days on balanced; ended 8/17/34
7 days on high carbohydrate; ended 8/24/34
7 days on balanced; ended 8/31/34

In the second experimental period following an interim of $2\frac{1}{2}$ months, during which the animals were maintained on the regular stock diet, the following scheme was used:

7 days on balanced; dog G replaced A; ended 11/7/34
7 days on high protein no. 2; ended 11/14/34
7 days on high protein no. 3; ended 11/21/34
7 days on high protein no. 2; ended 11/28/34; dog B became ill
7 days on high protein no. 4; ended 12/5/34
7 days on balanced; ended 12/12/34
7 days on high fat no. 2; dog H replaced B; ended 12/19/34
7 days on high fat no. 3; ended 12/26/34
7 days on high fat no. 2; ended 1/2/35
7 days on balanced; ended 1/9/35

Blood was drawn from the external jugular vein. Three grams of glucose per kilogram of body weight in 100 cc. of water were given by stomach tube; blood samples were drawn ½, 1, 2 and 3 hours after the glucose was given.

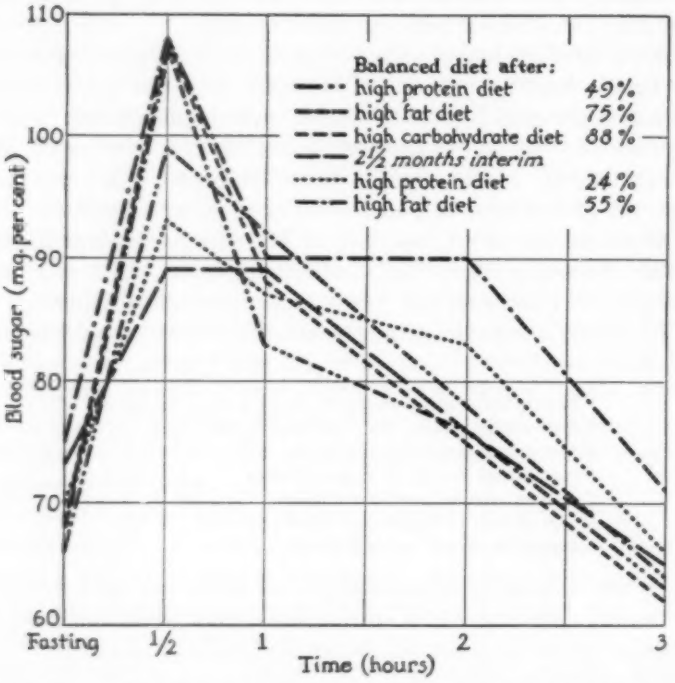

Fig. 1 Showing the residual effects of 24 and 49 per cent protein, 88 per cent carbohydrate, and 55 and 75 per cent fat diets; these are found after 7 days on the balanced diet, following the experimental diet.

Blood sugar was determined by the Shaffer-Somogyi ('33) method. All analyses were made in duplicate, using 1 cc. of blood for each. The average for the two determinations is used in the report. To prevent coagulation, potassium oxalate was used (2 drops of 20 per cent solution for each 3 cc. of blood). The blood was analyzed as soon as drawn, to prevent glycolysis.

RESULTS

In the six periods of balanced diet, 35 glucose tolerance tests were performed. The results are shown below.

	Fasting	*½ hour*	*1 hour*	*2 hours*	*3 hours*
18 tests on male dogs	71	91	84	75	64
17 tests on female dogs	69	108	93	83	65
Average of 35	70	99	88	79	65

It will undoubtedly be more instructive to report the results on each period of balanced diet separately, to show

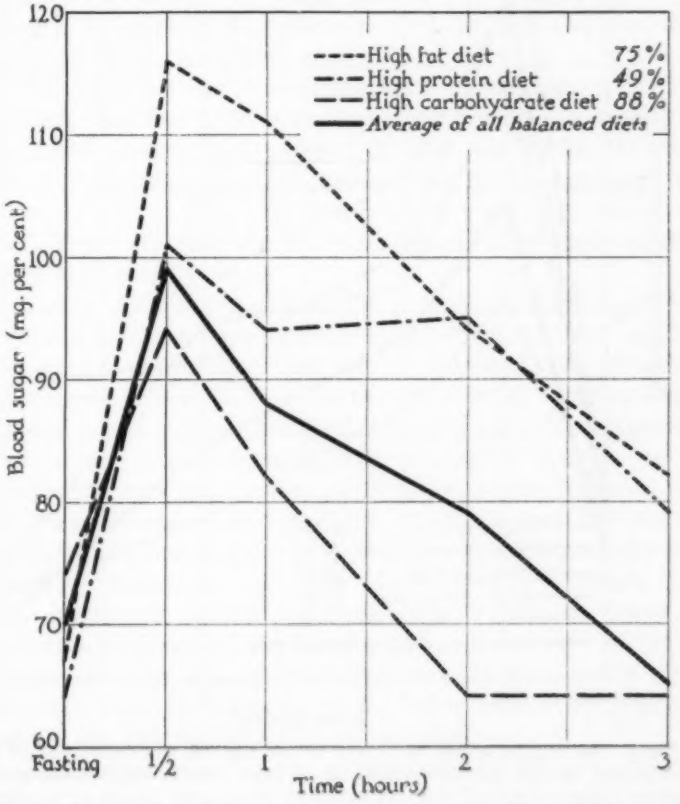

Fig. 2 Showing the effects of 75 per cent fat, 49 protein, and 88 per cent carbohydrate diets on the glucose tolerance of six dogs. First experimental period.

whether or not the effects of the experimental diets are still to be noticed, even after 7 days on the balanced diet. The average results are shown in figure 1.

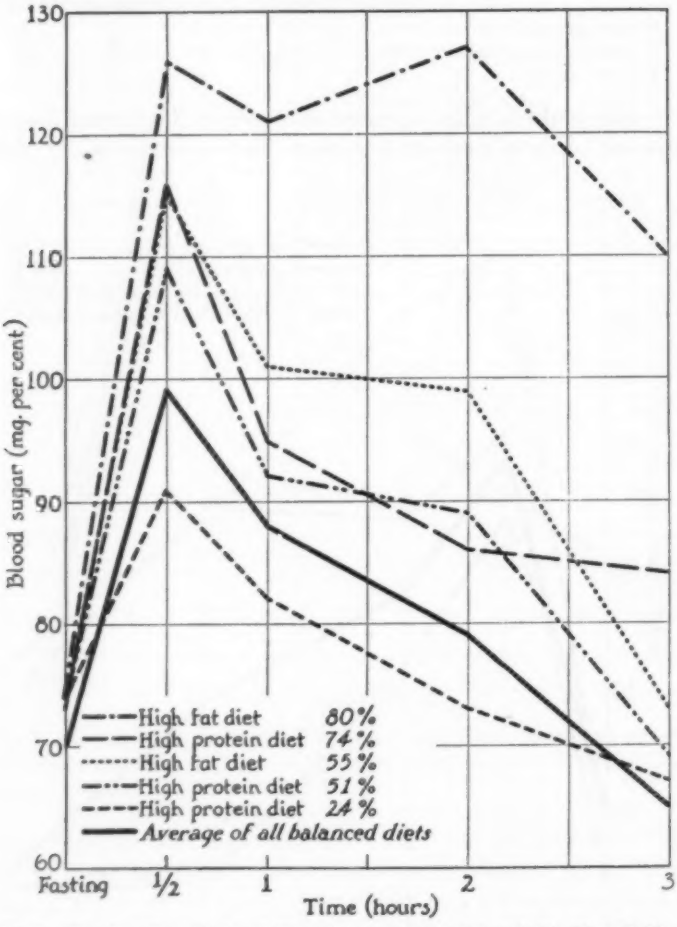

Fig. 3 Showing the effects of 55 and 80 per cent fat and 24, 51 and 74 per cent protein diets on glucose tolerance of six dogs. Second experimental period.

The results found on the high protein diet no. 1, on the high fat diet no. 1, and on the high carbohydrate diet are shown in figure 2; this covers the first experimental period.

The results found on the other experimental diets, used in the second period, are shown in figure 3.

DISCUSSION

In this dietary study an attempt was made to select materials which were suitable for dogs for the various diets. Since all were adult animals the aim was to hold the weight steady by giving the amount needed; the animals were weighed three times each week.

The balanced diet was relished by all the dogs; the high protein diet no. 1, in which granular gelatin was present, appeared distasteful to some of the dogs, but during that week the weather was unusually hot, so this factor may have had some influence. This was practically the only diet which was not completely eaten by all the animals. The high fat diets were very readily eaten.

During the first experimental period, the effects of high protein, high fat, and high carbohydrate diets were studied; the animal was placed back on the balanced diet after each experimental diet, before the next was tried. In the second experimental period, different percentages of high protein and high fat were studied, with a gradual increase to the maximum, and then a gradual decrease to the balanced diet.

It was necessary to use 3 gm. of glucose per kilogram of body weight for dogs, although 1 gm. per kilogram is sufficient for human beings. This was given by stomach tube; the passage of the tube did not seem to have an important effect on the level of the blood sugar; the average of fasting sugars for the six original dogs was 59 mg. per cent; 10 minutes after the passage of the tube, with introduction of water, the average was 62 mg. per cent.

In the tests after 7 days on the balanced diet, as shown in figure 1, there was little change in the fasting sugar, regardless of the type of diet which had been used before the

balanced. There is noted a tendency for the level at the half-hour to be higher, if high fat or high carbohydrate had been used before the balanced. This suggests that these diets leave some residual effect, which is noted at the half-hour period in the glucose tolerance test, even if a week of balanced diet has followed the experimental diet.

From figure 2 it will be noted that on high protein diet no. 1, there is a definite decrease in tolerance for glucose. This decreased tolerance is even more marked after high fat diet no. 1. The fasting level was slightly lower than the control, in both cases. With the high carbohydrate diet, the fasting level was higher, and the tolerance was increased; the results differed only slightly from those on the balanced diet.

In the second experimental period, as shown in figure 3, decreased tolerance after high protein was again noted; the higher the protein percentage in the diet, the greater the effect on the tolerance. The effect after 24.1 per cent protein was not noticeable.

With both 55 per cent and 80.1 per cent fat in the diet, a marked decrease in the tolerance for glucose was found.

From the results, one is justified in concluding that both increased protein and increased fat in the diet decrease the tolerance; in both cases, however, the carbohydrate in the diet is low.

There were great variations among the animals. Dog A showed the greatest effect as a decreased tolerance on the high fat diet. Dog B tended to show a delayed effect; this was most evident on the balanced diet after previous high fat and high carbohydrate diets. Dog C showed a delayed effect after the high fat diet; he showed a decreased tolerance on the high protein diets, but seemed gradually to overcome it. Dog D showed both an immediate and a delayed effect on the high protein and the high fat diets. She even showed a decreased tolerance on the high carbohydrate diet. Very great changes were noted in the blood sugar. Dog E behaved similarly to dog D. Dog F showed a decreased tolerance on

all the experimental diets. Dog G showed decreased tolerance on the high fat and high protein diets; the high carbohydrate was not done. Dog H showed a markedly decreased tolerance on high fat diets; this animal was a typical diabetic on the highest fat diet.

It is difficult to know how to interpret all the above findings. The differences in glucose tolerance are supposedly not due to differences in the rate of absorption. Trimble ('33) found glucose was absorbed from the gut of normal dogs at about the rate of 1 gm. per kilogram of body weight per hour.

Greenwald ('24) did not find a decreased tolerance after high protein diets, but did after high fat diets. du Vigneaud's ('25) findings agree with those of Greenwald. Malmros ('28) found low tolerance after low carbohydrate diets, and after high fat diets. He found that the decreased sensitivity toward glucose disappeared shortly after the return to the normal diet. He disagrees with other investigators in his results on high carbohydrate diets. Tolstoi ('29) found reduced tolerance after a high fat diet, with a return to normal tolerance within 2 weeks on a balanced diet. Sweeny ('27 and '28) found decreased tolerance after high protein and high fat diets, but increased tolerance after high carbohydrate diets. Himsworth ('33 and '34) found reduced glucose tolerance after high fat diets, and improved tolerance after a diet rich in carbohydrate. He considered the characteristic responses due to a change in the animal's susceptibility to insulin.

One should be exceedingly cautious in making general statements, since there is such variation in individual animals.

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ADEQUACY OF SIMPLIFIED RATIONS FOR THE COMPLETE LIFE CYCLE OF THE CHICK

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ONE FIGURE

(Received for publication July 31, 1935)

So far as we are aware Osborne and Mendel ('18) were the first to achieve even the slightest success in rearing baby chicks on simplified diets. They used rations similar to those that had been found adequate for the rat, but it is evident from their brief description that these diets do not make even a close approach to supplying the minimum requirements of the chick. It is clear now that their difficulties were partly due to the fact that vitamin D had not been recognized at the time their observations were taken, but the attempts made after vitamin D was discovered were only slightly more successful than those made before.

Hogan, Guerrant and Kempster ('25) presented evidence that the failures were due solely to nutritional deficiencies, and this view was confirmed by Hogan and Shrewsbury ('30). More recently Hogan and Boucher ('33) succeeded in formulating a simplified ration that is adequate for growing chicks. It is quite evident now that the rations commonly used when the rat is the experimental animal are grossly inadequate for the chick, for it was not until drastic changes were made in the vitamin components that we were able to rear normal chicks consistently on simplified diets. No other successful attempt has come to our attention.

[1] Contribution from the Missouri Agricultural Experiment Station journal series no. 429.

In our more recent work the chicks were discarded at 6 weeks, for our earlier experience had demonstrated that when a ration is deficient practically all the abnormalities will develop within 4 weeks. It is well recognized though that 6 weeks is too short a period to establish the complete adequacy of a diet, so in order to provide a more rigorous test an attempt was made to rear chicks through successive generations.

EXPERIMENTAL

The experimental chicks were single comb White Leghorns. They were reared in battery type brooders, but as they approached maturity they were placed in wire cages 2 feet square. The floors of the brooder were wire screens, but the cages had floors of sheet metal, covered with a litter of sand. Both the brooders and cages were located in rooms that were equipped with thermostatic heat controls. The chicks were weighed, transferred to the brooders, fed and watered when 1 day old. The rations are described in table 1.

As will be shown later the rations were changed at frequent intervals. This was because our simplified diet was being developed at the time this investigation started, and as it was modified from time to time corresponding changes were introduced in the rations of the chicks that were reared to maturity. Later on as it became evident that the fertility of the eggs was low, numerous changes were made in an effort to correct that condition, but there is no reason to believe the rations were improved thereby.

Cockerel 994 and hens 1243 and 1297 represented the first generation of the first trial. They were hatched September 1, 1931, and given ration 1405 at once. Both pullets began laying at an early age, one at 162, the other at 164 days. The hens were mated with the cockerel, but the fertility of the eggs was low, and none hatched. After it seemed certain that living chicks could not be obtained from this group, the male was mated with a hen from the poultry farm, and hen 1297 was mated with a male from the farm. Living chicks were obtained from both crosses, showing that fertile chicks of both

TABLE 1

Percentage composition of rations

	1405	1521	1552	1572	1601	1615	1668	1674	1713	1799	1801	1983	1996	2033
Casein[1]	35	35	35	35	35	20	20	35	35	35	35	35	35	35
Corn starch	35	17	29	29	30	45	42.6	27.6	30	28	27	21	19	25
Lard	3	5	7	7	11							5	5	
Milk fat						11	11	11	11	11	7	6	6	6
Cellophane	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Salts[2]	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Calcium carbonate							1.3	1.3						
Monosodium phosphate							1.1	1.1						
Tricalcium phosphate											1	1	1	
Cod liver oil	2	2	2	1	1	1	1	1	1	1	1	3	3	3
Wheat germ oil[3]				1	1	1	1	1	1	1	1	1	1	1
Acid hydrolyzed yeast[4]	5	8	5	5	4	4	4	4	4	4	4	4	2	2
Water extract of yeast													4	4
Ether extract of egg yolk	10	8	6	6	2	2	2	2	2	2	6	6	6	6
Purified liver extract[5]	1	8	5	5	5	5	5	5	5	6	6	6	6	6
Liver extract residue[6]	1	8	3	3	3	3	3	3	3	4	4	4	4	4
Tikitiki[7]	1	2	1	1	1	1	1	1	1	1	1	1	1	1

[1] Extracted with dilute acetic acid.

[2] Osborne and Mendel ('19).

[3] The ether extract of wheat germ.

[4] Prepared by hydrolyzing yeast with sulfuric acid.

[5] A commercial product used in the treatment of pernicious anemia. Supplied by Dr. David Klein of the Wilson Laboratories, Chicago, Ill.

[6] The fraction precipitated by 70 per cent alcohol during the manufacture of the Purified Liver Extract. See footnote 5 for source.

[7] Alcoholic extract of rice polishings, prepared by the method of Wells ('21).

sexes could be reared on the simplified diet, though the fertility was of a low order. The two chicks from hen 1297 were reared to maturity, and the pullet, no. 3472, laid her first egg at 152 days. She was mated with her full brother, no. 3471, and one chick was hatched, but it was subnormal in vigor and died the third day. When 202 days of age pullet 3472 became lethargic, and died 16 days later. Post-mortem examination[2] revealed a 152-gm. ovarian tumor. Doctor Durant, specialist in poultry diseases, informs us this condition is usually the result of a broken egg in the oviduct, and frequently occurs in commercial flocks. Death was, therefore, not necessarily the result of a nutritional deficiency nor of confinement under laboratory conditions. Until the time this tumor developed the hen was very active and indistinguishable from those reared normally. The male, no. 3471, was likewise normal and unusually belligerent. Both male and female had excellent plumage and very red combs and wattles.

Returning to the first generation group, all three were normal until March 22, 1932, when hen 1243 developed symptoms that resemble polyneuritis. The head retractions gradually became more severe, and death followed 8 days later. On July 20, 1932, mild head retractions were noticed in hen 1297, and this condition continued until September 2nd, when the observations on the first generation were discontinued. The decline in both these hens followed periods of active egg production, and the added demand during that period is believed to be the cause of the breakdown. The male, no. 994, was under observation continuously for 365 days. He received nothing but the simplified ration, was maintained constantly under laboratory conditions, and was normal throughout, except the toes were crooked.

After it became apparent that the first attempt to secure second generation chicks would probably fail, if the parent birds received simplified diets exclusively, a second trial was

[2] We are greatly indebted to Dr. A. J. Durant who made most of the post-mortem examinations.

started. The group included one male, no. 1862, and two females, nos. 1869 and 1880, and they were started at once on ration 1521. Hen 1880 began laying at 150 days of age, and laid a total of forty-eight eggs. Hen 1869 began laying at 154 days, and laid seventy-five eggs in all. Both hens were mated with cockerel 1862, and in each case living chicks were obtained. A total of eighteen were hatched and eleven survived. From this second generation, two males, nos. 101 and 4350, and two females, nos. 4347 and 4403, were then reared to maturity, in an attempt to obtain a third generation on a simplified diet.

When considered from all standpoints the record of hen 4403 is the best we have observed. She began laying at 170 days of age, and in the following 390 days she laid 249 eggs, a rate of 233 per year. However, she was obviously failing on the 568th day, and was killed when 585 days old. During the preceding 3 weeks she had lost approximately 600 gm. in weight. It developed at autopsy that a broken egg in the oviduct was the immediate cause of the decline.

In the meantime twenty-one of her chicks had been reared, and three were retained until they were mature. One of the females, no. 99, laid her first egg at the age of 169 days, and in the next 24 days she laid fifteen more. There was some indication when 181 days old that this pullet was failing, and she was killed on her 211th day. On post-mortem examination it was found that the duodenum was packed with sand. The other female, no. 100, was mated with a brother, no. 96, but the first attempt to secure living chicks failed. Few of the eggs were fertile and none hatched. It was thought the fertility of the male may have been of a low order, so he was replaced by a Rhode Island Red from the University Poultry Farm. Four living chicks were obtained from this cross and they were strong, vigorous, and grew rapidly on one of the simplified diets. They were discarded when 10 weeks old. The female was then remated with no. 96 and following this three eggs hatched. The chicks appeared normal at hatching and were above the average weight, but none survived longer

than 3 days. It is not believed that these deaths alone afford any positive evidence that the ration consumed by the mother was inadequate, as the conditions at that time were exceedingly unfavorable. The eggs were in the incubator during the last of July and first part of August, 1934, during the period of abnormally high temperatures. It is well recognized by poultrymen that chicks hatched during the summer are usually inferior. This hen began to decline at the age of 510 days, and died on her 528th day. The immediate cause of death was pneumonia, but the hen was excessively fat. Fatty degeneration of the liver, heart, and spleen were reported.

The male, no. 96, was killed and autopsied at the same time. He too was excessively fat, and the layer of adipose tissue in the posterior part of the body cavity was described as fully 1 inch thick. The liver had small indentations on the surface, but was not strikingly abnormal. The other organs seemed entirely normal.

The growth curves of the chicks retained until maturity are shown in figure 1, and their pedigrees are shown in table 2. The ration changes are shown in table 3.

In figure 1 the control weight-age curves were plotted from data published by Buckner, Wilkins and Castle ('18). Their chicks were reared under normal conditions on normal rations, and they grew more rapidly than any others reported, when corresponding numbers were involved. It is evident that, judged by any standard, the experimental chicks have grown at a satisfactory rate. The males grew normally and were apparently healthy throughout the period of observation. The females were normal until after they had been laying for varying intervals, but four out of six eventually developed some disease from which they did not recover.

Our chief interest was in the ability of the experimental chicks to reproduce, so a complete summary of the laying and incubation records is shown in table 4.

Even under the most favorable circumstances there is considerable variation in the fertility and viability of eggs, so

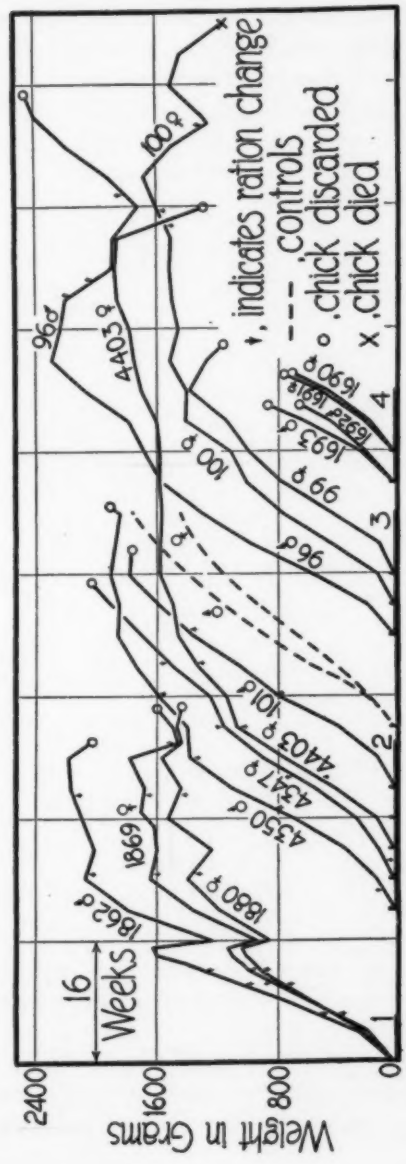

Fig. 1 Age-weight curves of four successive generations reared on simplified rations. The order in which they came is indicated by numerals at the base of the chart.

in one of the early trials it was decided to place commercial hatching eggs in the incubator as a control. These are compared with the total of experimental eggs in table 5.

Taking the data at their face value it must be concluded that the degree of success in rearing successive generations on the simplified diet was somewhat qualified. The fertility

TABLE 2

Relationship of chicks reared on simplified rations

GENERATION			
1	1869F (2–1–32)[1]	×	1862M (2–1–32) × 1880F (2–1–32)
2	4347F (10–24–32) 4403F (11–21–32)	×	4350M (10–24–32) 101M (12–24–32)
3	99F (6–16–33) 96M (7–20–33)	×	100F (6–16–33) × 1574M[2]
4	1, 2, 3 (8–11–34)[3]		1690F, 1691F, 1692M, 1693M (6–16–34)[4]

[1] Figures in parentheses indicate data of hatching.
[2] Rhode Island Red from Poultry Farm, reared on stock ration.
[3] None of these three survived longer than 3 days.
[4] All grew normally, were discarded at 10 weeks.

Autopsy record

1862M, killed 12–15–33. Normal.
1869F, 1880F, killed 1–13–33. Excessively fat, otherwise normal.
101M, killed 7–25–33. Normal.
4350M, killed 10–28–33. Normal.
4347F, killed 7–25–33. An 'internal layer.' May have been due to injury, as a broken egg. Twelve yolks were found. All organs normal.
4403F, killed 6–29–34. Decline due to broken egg.
96M, killed 11–25–34. Excessively fat. Liver had small indentations on surface. otherwise normal.
99F, killed 1–13–34. Duodenum impacted with sand.
100F, died 11–25–34. Fatty degeneration of liver, heart, spleen. Pneumonia immediate cause of death.

of the experimental eggs was less than half that of commercial eggs, though there was no marked difference in the hatchability of the fertile eggs.

It is impossible to determine from the available data whether or not the qualitative makeup of the ration was in any way responsible for the lowered fertility. Practically all

TABLE 3

Periods simplified rations were used

DAYS[1]	RATION	DAYS[1]	RATION	DAYS[1]	RATION
Chicks 1862, 1869, 1880		Chicks 4347, 4350		Chick 96	
1	1521	1	1668	1	1801
45	1552	17	1674	309	1983
72	1572	94	1713	323	1996
113	1601	140	1799	401	2033
173	1615	199	1801		
247	1668				
Chick 101		Chick 4403		Chicks 99, 100	
1	1713	1	1674	1	1801
109	1799	66	1713	343	1983
186	1801	112	1799	357	1996
		171	1801	435	2033
				Chicks 1690, 1691, 1692, 1693	
				1	1996

[1] Age when the corresponding ration was first supplied.

TABLE 4

Egg records of hens reared on simplified rations

HEN NO.	FIRST GENERATION		SECOND GENERATION		THIRD GENERATION	
	1869F	1880F	4403F	4347F	99F	100F
Days under observation	297	297	585	274	211	528
Age first egg laid, days	154	150	170	...	169	177
Number of eggs laid	75	48	249	...	16	90
Number of eggs incubated	73	48	135	...	0	64
Number of eggs fertile	28	20	64	...		17
Number of chicks hatched	7	11	31	...		7
Number of chicks reared	5	6	21			4

TABLE 5

Fertility and hatchability of experimental and of commercial eggs

	SOURCE OF EGGS	
	Experimental	Commercial
	per cent	*per cent*
Fertility	40.3	89.0
Hatchability of fertile eggs	43.4	50.6

of the older birds were excessively fat, and this decreases the efficiency of breeding stock. There is also some uncertainty as to the effect of close confinement, and indoor conditions, on fertility. All the males have enormously developed combs and wattles, and this may be a by-product of some physiological disturbance of unexpected significance. Buckner, Insko and Martin ('32) made a detailed study of the effects of confinement on the growth and development of male chickens. They observed that when the cockerels were reared indoors their combs were about twice the normal size, the testes were about half the normal size, and their fertility was low. These authors also mention the fact that lack of exercise is not conducive to high fertility. Two years later Buckner, Martin and Insko ('34) emphasized again the depressing effect of close confinement on male fertility, and included some observations on the effects of closely confining the pullets. The hens that were reared in confinement were less productive than the controls, and their eggs were below the average weight. The control group with the highest production laid an average of 202 eggs their first laying year. There were no significant differences between the experimental and control hens in fertility or hatchability. The percentage of mortality was very heavy among the confined hens, as 58.5 per cent of them died. If the data of these investigators are taken as a standard of performance during confinement, then the adequacy of our simplified ration would seem to be demonstrated beyond question. According to this standard our chicks on simplified diets grew rapidly, were almost entirely free from abnormalities, and their egg production was satisfactory. Our best hen, no. 4403, laid at the rate of 233 eggs per year, and this is 15 per cent higher than the average production of the best laying controls reported by Buckner, Martin and Insko ('34).

The reports just cited make it seem probable that close confinement alone could explain our most serious difficulties, but as a matter of fact there were some additional complications. For example it is recognized by poultrymen that the

fertility and viability of eggs is highest in the spring. It was necessary for us to incubate the eggs throughout the year, and it was often necessary to hold them unreasonably long before placing them in the incubator. Furthermore, the original line had of necessity been closely inbred, and a genetic factor may have been partly responsible for the later failures.

It has been mentioned that the only serious difficulty encountered was the low fertility of the third generation, and the delay in obtaining the fourth generation chicks. At this same time our studies with growing chicks were still under way, and we were perplexed to find that the simplified diet had suddenly become less reliable. A majority of the chicks grew normally, but there was a definite increase in the incidence of leg weakness indicated by enlarged hocks, crooked leg bones, or slipped tendons. Many of the affected chicks were also subnormal in weight. We had previously regarded this ration as almost infallible, for during the preceding 2 years approximately 200 chicks were reared on this, or similar rations, with uniform success. The mortality had been 7 per cent, and the abnormalities of all types 8.1 per cent. The unexpected failures with growing chicks seemed to leave no doubt that the ration had become defective in some way, and it is reasonable to suppose that this weakness also had an adverse effect on the reproductive capacity of the mature individuals.

The source of these disturbances is not clear as yet, but various possibilities have been considered. It is well recognized that cod liver oil is toxic at times, and the supply then in use may have been inferior. A particular preparation of one of the other vitamin supplements may have been defective. A change in one of the original materials may have had an unfavorable effect on the inorganic constituents, especially of calcium and phosphorus, and for the present we have adopted this as our working hypothesis. Hunter and Funk ('30) stated some time ago that a maladjustment of these two elements increases the incidence of leg weakness, and this

observation has been confirmed repeatedly. Preliminary studies of the mineral content indicate that our recent difficulties will be explained in the same way.

In the course of our efforts to further standardize the preparation of the basal diet, various rations seemed promising. One of these, no. 2159, is described below.

Ration 2159

Casein	35	Wheat germ oil	4
Cornstarch	30.5	Acid hydrolyzed yeast	4
Cellophane	3	Liver extract	6
Lard	11	Tikitiki	2
Salt mixture[3]	4	$CaCO_3$	0.5

In addition each chick receives daily 0.2 mg. crystalline carotene,[4] and 240 International units of irradiated ergosterol.[5]

As made up at present ration 2159 contains 0.7 per cent of calcium and 0.7 per cent of phosphorus. It has been used twice, and altogether twenty-four chicks received it as their sole diet. There were no mortalities and the rate of growth was satisfactory but there were two cases of slipped tendon, a rate of 8.3 per cent. Two or three similar rations have been equally satisfactory, and we feel they may be used with considerable confidence. We see no reason to doubt, therefore, that these simplified diets contain all nutrients that are required by the chick. It may be that some of them contain some nutrient in insufficient amount, or there may be some maladjustment between them. However, it is our view that the fundamental difficulties have been overcome. It may be an exacting task, but the way is open to determine qualitatively and quantitatively the nutritional requirements of the chick.

[3] Osborne and Mendel ('19).
[4] Purchased from S. M. A. Corporation, Cleveland, Ohio.
[5] Kindly supplied by Dr. C. E. Bill of Mead Johnson and Company.

SUMMARY

1. Four successive generations of chicks have been reared on simplified rations.

2. All chicks grew rapidly. The males were normal in appearance throughout the period of observation but their fertility was low. The females were normal until they attained maturity, and their egg records compared favorably with those obtained under normal conditions. After periods of intensive egg laying the mortality of the hens was high.

3. Data published by other investigators indicate that the low fertility of the males and the high mortality of the females can be explained by the fact they were reared under laboratory conditions. If this explanation is correct the simplified diets are complete in all respects.

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THE EFFECT OF 1 PER CENT COD LIVER OIL ON THE RAT, WITH PARTICULAR REFERENCE TO THE THYROID GLAND

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Hamilton ('34) in comparing the thyroid weights of his control rats with the series of Freudenberger ('32) found that his weights were significantly lower. The stock diet fed these two series of animals differed only in that 1 per cent cod liver oil had been added between the time Freudenberger made his study and the time Hamilton did his work. Since Thomson ('32) found that 1 per cent cod liver oil was not protective against the goitrogenic activity of rachitogenic diets, Hamilton concluded that the marked variation between Freudenberger's series and his own was due chiefly to differences in technic of dissection. He felt that the possibility of actual changes in the thyroid weight of the colony in such a short time was rather slight.

Freudenberger and Billeter ('35) noted that the thyroid weights of their 6-month-old rats were smaller than those of Freudenberger's ('32) 3-month series. Freudenberger and Billeter were feeding their rats the same diet used by Hamilton.

These two instances led us to believe that the small thyroid weights might actually be due to the addition of the 1 per cent cod liver oil to the diet so this experiment was carried out to test this possibility.

MATERIALS AND METHODS

Wistar albino rats of the female sex were used. They were weaned at 3 weeks of age and at once put on the diets. Twenty-five were given a constant supply of a well-balanced stock diet consisting of: casein, 15 per cent; whole milk powder, 10 per cent; sodium chloride, 0.8 per cent; calcium carbonate, 1.5 per cent; butter (unsalted), 5.2 per cent; and whole ground wheat, 67.5 per cent. Twenty-five litter mate controls were fed the same diet with the exception that 1 per cent pure cod liver oil (U.S.P.) was added. The diet was made up fresh at least every other day. All the animals were given excellent care which was similar in all cases. The care given was similar to that described fully by Freudenberger ('32).

The progress of each animal was carefully watched, and each animal was weighed weekly. At 4 months of age the animals were killed and autopsied according to the method of Jackson ('30). Both the nose-anus and tail lengths were measured. The head was weighed as well as the suprarenal glands, hypophysis, thyroid, thymus, and ovaries. The organs removed were fixed in Bouin's fluid. Sections of the thyroid gland, 10 μ in thickness, were stained with hematoxylin and eosin.

A comparison of the weights and measurements of the two groups of animals was made using modern biometrical methods. As is customary the difference between two means divided by the probable error of the difference is spoken of as the significance ratio in this paper.

OBSERVATIONS

Growth in body weight

The growth of the two groups of rats was practically identical. Those not given cod liver oil averaged 36.8 gm. in weight on the day the experiment was begun while those given the oil averaged 36.1 gm. The average weekly weights of the two groups were practically the same throughout the

experiment. At the time of autopsy (table 1) the rats which were not given oil averaged 190.8 gm. in weight while those on oil averaged 191.1 gm. This slight difference is insignificant, the significance ratio being only 0.11. The addition of 1 per cent cod liver oil to the diet apparently does not affect growth in body weight.

TABLE 1

Statistical summary of results

	MEAN		DIFFERENCE BETWEEN OIL AND NO OIL MEANS	DIFF. / P.E. DIFF.
	Oil	No oil		
Body weight (gm.)	191.1 ± 2.3	190.8 ± 1.7	+ 0.3 ± 2.9	0.11
Nose-anus length (cm.)	19.91 ± 0.06	19.94 ± 0.06	— 0.04 ± 0.09	0.42
Tail length (cm.)	17.96 ± 0.11	17.93 ± 0.09	+ 0.03 ± 0.15	0.19
Head (gm.)	17.28 ± 0.14	17.37 ± 0.10	— 0.09 ± 0.17	0.52
Suprarenal glands (gm.)	0.0453 ± 0.0009	0.0466 ± 0.0008	— 0.0013 ± 0.0013	1.05
Hypophysis (gm.)	0.0105 ± 0.0003	0.0107 ± 0.0002	— 0.0002 ± 0.0003	0.54
Thyroid (gm.)	0.0169 ± 0.0002	0.0203 ± 0.0004	— 0.0034 ± 0.0004	7.72
Thymus (gm.)	0.4755 ± 0.0110	0.4610 ± 0.0092	+ 0.0145 ± 0.0144	1.01
Ovaries (gm.)	0.0578 ± 0.0011	0.0579 ± 0.0012	— 0.0001 ± 0.0016	0.07

Nose-anus length

The rats not given oil had a final average nose-anus length (table 1) of 19.94 cm. while those which received the oil had an average length of 19.91 cm. The significance ratio for this slight difference is 0.42 so the difference must be regarded as insignificant.

Tail length

The tail length (table 1) of the rats not fed oil averaged 17.93 cm. while those fed the oil had an average length of 17.96 cm. The significance ratio for this small difference is only 0.19 so the difference is not significant.

Occurrence of infections

Although the gross appearance of the lungs was carefully noted in each case, practically no evidences of pathological changes were noted. In the case of one rat receiving the oil a small solid area of lung tissue was noted. The lungs of all the other animals appeared entirely normal.

The tympanic cavities were examined for pus. Pus was noted in one or both ears of eighteen of the rats not receiving the oil. Similar observations were made in the case of sixteen rats receiving the oil. The presence of the oil in the diet apparently did not significantly change the susceptibility of the rats to middle ear infection.

No other infections were noted in any of the rats studied.

Head weight

The average weight of the head (table 1) of those rats not being fed oil was 17.37 gm. while the average weight of those being fed oil was 17.28 gm. Since the significance ratio for this difference is only 0.52, the difference can be regarded as being insignificant.

Weight of individual organs

Suprarenal glands. The suprarenal glands (table 1) of those animals not receiving oil averaged 0.0466 gm. in weight while those of the animals receiving oil averaged 0.0453 gm. This difference is also insignificant since the significance ratio for the difference is only 1.05.

Thyroid. The average weight of the thyroid gland (table 1) in those animals not receiving oil was 0.0203 gm. while the average weight of the thyroid in those animals receiving oil was 0.0169 gm. The thyroid is, therefore, smaller in those animals receiving 1 per cent cod liver oil in their diet. The difference is unquestionably significant since the significance ratio is 7.72. The histological structure of the thyroid was studied in six animals of each group. No definite differences could be detected. Since such a great variability exists in

the normal histological structure of the rat thyroid, as has been recently pointed out by Coplan and Sampson ('35), it is extremely difficult to detect histological differences between the thyroid glands of two series of rats unless they are very well marked.

Hypophysis. No significant difference was found between the average weights of the hypophysis (table 1) of the two series of rats. The hypophyses of the rats not receiving oil averaged 0.0107 gm. in weight while those of the rats receiving oil averaged 0.0105 gm. The significance ratio for this difference is only 0.54.

Thymus. The average weight of the thymus (table 1) in those animals not receiving oil was 0.4610 gm. while that of those receiving the oil averaged 0.4655 gm. Since the significance ratio for this difference is only 1.01, the difference cannot be considered significant.

Ovaries. The average weights of the ovaries (table 1) of the two groups of rats were practically identical being 0.0579 gm. in the case of those not receiving oil and 0.0578 gm. in those receiving oil. The significance ratio is only 0.07.

DISCUSSION

It is interesting to note that the addition of 1 per cent cod liver oil to a normal well-balanced diet has no effect on growth in body weight or length, weight of head, weights of the suprarenal glands, hypophysis, thymus, or ovaries, but results in a significantly smaller thyroid. The incidence of infections was also the same in the two groups of rats. There would seem to be no evidence of a vitamin deficiency in the diet used even without the oil. Growth was good and the animals were remarkably free from all infections except of the middle ear. There was no evidence of xerophthalmia.

Sherwood, Toth and Carr ('34) found that feeding 1 to 5 cc. cod liver oil per kilogram body weight per day to rats produced a depletion of the colloidal content of the acini. They showed that an excess of vitamin D had no effect on the thyroid gland. Sherwood and Luckner ('35) found that ex-

cessive amounts of vitamin A in the diet of rats caused an irregular distribution to a depletion of the colloid of the thyroid gland. The cells lining the acini increased in height and a marked increase in stroma occurred. They found that iodine present in cod liver oil was not responsible for any change in the thyroid gland, but that iodine in the approximate amount found in cod liver oil, given in the form of potassium iodide produced an increase in colloid after 18 days. The acini were strikingly distended and composed of extremely low epithelium.

Coplan and Sampson ('35) found that an iodine deficiency in the diet of albino rats produced an initial hypertrophy of the thyroid gland in both males and females and that this persisted for a longer time in females. It was followed in both sexes by atrophy. They found that vitamin A deficiency produced definite hypertrophy in the female, but consistent atrophy in the male. A deficiency of iodine and vitamin A in the diet produced an initial hypertrophy in males and females. In males atrophy followed with continued deficiency. Coplan and Sampson have reviewed the literature in regard to the effects of diets deficient in iodine on the thyroid gland. They also call attention to the literature in regard to other dietary defects (vitamin A, vitamin D, excess of calcium) on the thyroid gland. Since this has been done so recently very brief mention of the literature will be made in this paper.

As far as we are aware there is no evidence in favor of our diet without the cod liver oil being deficient in either vitamins or iodine. It is, therefore, interesting to note that the addition of 1 per cent cod liver oil to the diet resulted in a smaller thyroid gland with no other changes that could be detected in the animals. It would seem that the thyroid gland is the organ which is most sensitive to cod liver oil. Agduhr ('29) showed that regressive tissue changes were produced with large doses of cod liver oil. It is, however, clear that no such changes were produced in this experiment.

All experimental workers using albino rats are very interested in normal organ weights. The results in this experi-

ment bring up the interesting question of just what is the normal thyroid weight. Is it the weight as found in those rats without the oil or that of those which received oil in their diet?

SUMMARY

Twenty-five female Wistar albino rats have been fed a well-balanced stock diet from 3 weeks of age to 4 months of age while a similar group has been fed the same diet with 1 per cent cod liver oil added. All the animals were killed and autopsied at 4 months of age. No difference was found between the groups in body growth, body weight, nose-anus length, tail length, incidence of infections, or in the weights of the head, suprarenal glands, hypophysis, thymus or ovaries. The thyroid gland was found to be significantly smaller in those rats which received the diet containing the cod liver oil.

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THE RELATION OF THE RATE OF GROWTH TO DIET [1]

III. A COMPARISON OF STOCK RATIONS USED IN THE BREEDING COLONY AT THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION

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ONE FIGURE

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During the last 20 years, numerous workers have reported progressive increases in the average growth rate of the albino rat in response to selective breeding or to improved dietary conditions. References to some of these observations have been made by Osborne and Mendel ('26) and by Anderson and Smith ('32).

It is well known that the animals in the colony at the Connecticut Agricultural Experiment Station have been bred for about 25 years without accessions from elsewhere. Any changes in rate of growth or general condition of the rats during this period should, therefore, be attributed to the management of the colony. In 1926, in connection with a study of the factors that influence the rate of development, Osborne and Mendel ('26) recorded growth curves for males of the colony for 1912, 1919 and 1925. They also showed that rats fed a special ration, relatively high in protein, grew at the rate of 4.0 gm. a day for the period from weaning to

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington.

100 days of age, as contrasted with 2.1 gm. for stock males. This observation, followed by the work of Anderson and Smith ('32), led to the adoption of a new stock ration which has been in use since 1931. The accelerated growth rate, first observed by Osborne and Mendel for a few animals, has been maintained as a regular characteristic of the colony for more than ten generations. It seems of interest, therefore, to record at this time some of the data regarding the animals of the colony as it is today, together with a brief account of its history prior to 1931.

From the dietary standpoint, we recognize three periods, and for convenience designate them as 1912, 1919 and 1935. For the first of these periods, 1912, the stock ration consisted of an abundance of 'Old Grist Mill' dog biscuit and sunflower seeds, with carrots and other fresh vegetables two or three times a week. Rock salt was always kept in the cage. After October 1912, nursing mothers and young rats received fresh milk or 'milk food' in addition. The records do not indicate how long the milk was supplied after the young were weaned. During the second period, beginning in 1915, a special brand of dog biscuit[2] was used. This was supplemented with fresh vegetables, usually carrots. Nursing mothers and young rats under 4 or 5 weeks of age received in addition a 'milk food' paste consisting of milk powder 60 per cent, starch 12 per cent, and lard 28 per cent (Ferry, '20). The ration in use now is a modification of that devised by Anderson and Smith as a basis for their study of the effect of mating interval on reproductive performance (unpublished data). It consists of G.L.F. calf meal (Maynard, '30) 97 per cent and cod liver oil 3 per cent.[3] Nursing mothers and young rats under 6 weeks of age receive in addition a 'paste food' consisting of casein 25 per cent, whole milk powder 25 per cent, wheat embryo 20 per cent, and lard 30 per cent.

[2] Special dog biscuit composed of wheat, oats, beef, rice, corn-meal and cotton-seed oil, made by Potter-Wrightington, Inc., Boston.

[3] The formula has been modified recently in accordance with a change in the commercial calf meal. This product now contains 5 parts in 2000 of "cod liver oil reinforced in vitamin D."

The calf meal food and 'paste food' are both supplied ad libitum. Each rat also receives 1 gm. of dried yeast daily except Sunday, and those without 'paste food' are given 3 gm. of wheat embryo per week. No 'green' food of any sort is used.

Rats are first bred at 120 days of age, according to the general procedure described by Ferry ('20). Each female is permitted three matings, with an interval of 3 weeks between weaning and subsequent remating. Litters are reduced to eight rats at birth, and the young are weaned at 21 days of age. The stock colony is maintained by selecting young from first, second or third litters. No female is mated more than once with the same male. Our records include the number of young cast, weights of the young at birth, at weaning, and at weekly intervals thereafter until the animals are terminated. Unfortunately, for the sake of comparison, the earlier records are not so detailed. However, we have been able to secure from them data which may be considered representative of the earlier dietary procedures. The significant facts are summarized in figure 1 and table 1.

Figure 1 records the growth of male rats from weaning to 280 days, and of females until they are mated at 120 days. For both sexes there was a gradual increase in growth rate from 1912 to 1925. This was probably due both to improvement of the stock through careful breeding, and to the inclusion in the ration after 1912 of the 'milk food,' which was doubtless more easily utilized by the young animals. In the light of the very successful experiments of Osborne and Mendel in 1926, in which growth was accelerated by the use of diets that contained a higher percentage of protein than had previously been considered desirable, we may also attribute the increased growth rate in 1919 and 1925 over that in 1912 to the fact that the rations after 1915 supplied an increased proportion of total calories from protein, approximately 18 per cent as contrasted with 13 per cent in the ration used in 1912. Whether the more marked change in growth noted between 1925 and 1935 can be attributed to a further

increase in protein has not been fully determined. In the present ration, approximately 23 per cent of the total calories is supplied by protein. The marked acceleration in weight increase occurs in the period from weaning to 100 days of age. Subsequently the 1925 and 1935 curves are almost identical.

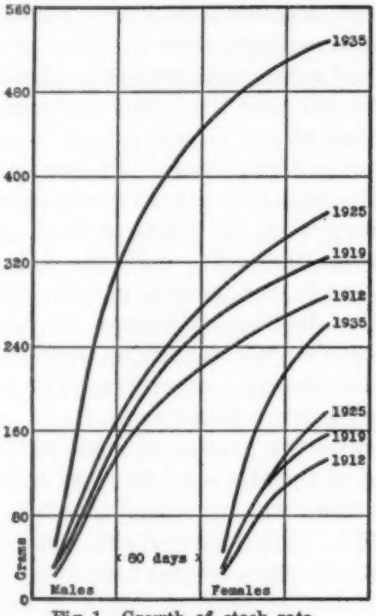

Fig. 1 Growth of stock rats.

TABLE 1

Summary of reproductive performance under different dietary conditions

	PERCENTAGE OF FERTILE MATINGS	NUMBER CAST	WEIGHT AT BIRTH	PERCENTAGE WEANED	WEIGHT AT WEANING		DAILY GAIN TO 100 DAYS	
					Males	Females	Males	Females
			gm.		*gm.*	*gm.*	*gm.*	*gm.*
1912	86	7.2	...	71	23	26	1.8	1.2
1919	65	6.3	...	67	31	31	2.0	1.4
1925	68	6.4	...	76	31	30	2.1	1.6
1935	93	9.6	5.8	90	48	47	4.0	2.5

Figures are averages of rats in the colony during the year indicated.

We have been inclined to attribute the rapid development during the first 6 weeks of life to the use of 'paste food' for the nursing mothers and young rats. That this is one factor is shown by a comparison of our weight gains with those reported by Anderson and Smith ('32) who used the same combination of calf meal food and 'paste food,' but with the latter available at all times, instead of only during the first 6 weeks. On this regimen, their rats maintained the increased growth rate over longer periods than do our animals. These authors recorded the average time for male rats to grow from 60 to 200 gm., to 300 gm., to 400 gm., and to 500 gm., respectively. The gain to 200 gm. was made in 23.3 days; our animals require 25 days for the same increase. With an average weaning weight of 48 gm., our rats would receive 'paste food' for only 19 or 20 days out of the total period required to make this gain. For the second interval, from 60 to 300 gm., the difference is more marked, namely 41.1 days for the Anderson and Smith rats, while our time is 51 days. For the two other intervals indicated, the gap is still wider, being 69.1 days as contrasted with 100 days for the gain from 60 to 400 gm., and 102.4 days in comparison with 197 days to attain the maximum weight discussed in their report.

The use of a 'paste food' is not the only factor, however, which may be regarded as responsible for the superior rate of growth in this colony in 1935 as compared with former years. A somewhat similar concentrated food was in use from 1912 until 1931 when the present regimen was initiated. The 'paste food' in use now supplies more protein than did the old 'milk food' and also includes 20 per cent of wheat embryo. A limited number of observations indicates that the calf meal food is itself in part responsible for the change in growth rate. We have observed the growth of male rats without the use of 'paste food' subsequent to weaning. Despite the fact that such growth is inferior to that observed with the more complex ration, it is considerably above that obtained under the old dog biscuit-milk food regimen. We feel, therefore, that both the calf meal food and the paste food contribute

to the present improved rate of growth. According to our analyses, this ration is richer in protein than the older one, and definite amounts of dried yeast and wheat embryo are fed separately, thus ensuring, with the cod liver oil included in the calf meal food, a liberal supply of 'accessory food substances,' even though no 'green' food is used.

Data for the colony at different periods in its history, summarized in table 1, afford evidence of the effectiveness of the present ration for reproduction, as judged by the number and size of litters, percentage weaned, and weights of the young at birth and at weaning. The figures for 1912 indicate a better record for number of young cast and weaned than in the years immediately following. It will be noted, however, that the animals were small at weaning. In the early records, there is frequent mention of the fact that nursing mothers were given milk, and bread and milk, "to see if the young would grow any better." That such additions were successful is shown by the fact that after 1912 fresh milk or 'milk food' was supplied regularly to nursing mothers and young rats. As a result, the young attained weights at weaning which were appreciably better as is shown by the 1919 record. During this same period, however, there was a reduction in the percentage of fertile matings. We have no explanation for this decrease.

We have included data for 1925 in the table in order to show the behavior of the stock animals at the time the rapid growth studies were initiated (Osborne and Mendel, '26). It is clear that there had been no marked increase in number and size of litters, or in rate of growth, as a result of careful selection of vigorous animals for breeding. If the type of growth now observed had been induced largely through selection, we feel that the upward trend would have been more marked in 1925, after the ration had been in use for 10 years. After the adoption of the new stock food in 1931, however, there was a marked improvement in reproductive performance and in rate of growth. These changes were evident immediately and have been maintained through succeeding generations.

SUMMARY

A comparison has been made of growth and reproductive performance of the albino rat during three periods in the history of the colony at the Connecticut Agricultural Experiment Station. A gradual increase in rate of growth has been noted during the entire 25 years. This increase was very marked when a change of ration was made in 1931. The improved growth rate has been accompanied by superior reproductive performance.

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THE COMPARATIVE RACHITOGENIC PROPERTY OF OATS AND CORN [1]

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The faulty skeletal development and poor teeth of animals (chiefly dogs) fed cereal diets were first emphasized by E. Mellanby ('18) and May Mellanby ('18). Later work led E. Mellanby ('26) to postulate the existence in cereals of definite rachitogenic substances or antivitamins which he called toxamins, the exact nature of which has not yet been determined. Mirvish ('29, '30) concluded that he had been able to concentrate a rachitogenic toxamin from cereals, especially from oatmeal. He separated an alcohol soluble fraction which lowered blood calcium when injected into the circulation of mature rabbits. King and Hall ('31), using chicks, also have concluded that oatmeal probably contains a specific rachitogenic substance.

The rachitogenic effect of cereals, especially oats, has been explained on other grounds than the presence of toxamin. Fine ('30) concludes that any difference between cereals is due to difference in vitamin D content, while Bruce and Callow ('34) believe that the inferiority of oatmeal is due to the lower availability of its phosphorus, present chiefly as salts of phytin (inositol hexaphosphate). However, Harris and Bunker ('35) using rats, failed to find any correlation between the rachitogenic properties of different samples of corn and either the absolute or relative amounts of phytin phosphorus present.

[1] This paper was prepared by Leroy S. Palmer from the thesis by Lawrence L. Lachat, presented in partial fulfillment of the requirements for the Ph.D. degree, University of Minnesota, 1933. Published with the approval of the director as paper no. 1372, journal series, Minnesota Agricultural Experiment Station.

On the other hand Booth, Henry and Kon ('35) report data for rats fed rachitogenic corn rations which are believed to support the conclusions of Bruce and Callow.

Steenbock, Black and Thomas ('27, '30) in extensive studies with rats and dogs failed to secure evidence of greater rachitogenic influence of oats than of corn, although both of these cereals seemed to be less antirachitic than wheat. de Wildt and Brouwer ('32), likewise, found corn to be more rachitogenic than oats, and more so than barley, rye or wheat. Steenbock and Kletzien ('31) were unable to isolate from cereals any material possessing rachitogenic properties.

The data of King and Hall ('31) are the only ones which have been interpreted in favor of the existence of rachitogenic substances in cereals which lend themselves to statistical treatment. Such a study of the data from their birds receiving the basal ration and their birds receiving the basal ration plus 20 per cent oat groats shows high variability in both groups and only a moderately significant difference between the means of the two lots ($P = 0.05$–0.02). The importance of this value is decreased somewhat because of poor control of other factors in the rations fed, other than a possible unidentified toxamin.

EXPERIMENTAL

This paper presents certain phases of a study of the comparative rachitogenic property of oats and corn. The experiments reported include, 1) a repetition of certain of the King and Hall experiments with chicks, in comparison with rations containing a higher proportion of oats and in which all important factors were controlled except the possible toxamin, 2) attempts to concentrate a rachitogenic substance from oat groats and demonstrate its activity, 3) tests of rachitogenic diets indicating that the severity of the rickets produced has a bearing on the demonstration of rachitogenic differences between oats and corn. Both rats and chicks were employed because of the recognized quantitative differences between the etiological factors concerned in the production of rickets in

these species. The relatively greater requirement for vitamin D by chicks than by rats was considered advantageous and the close analogy between infantile rickets and rat rickets was considered justification for employing rats in this study.

The chicks used were day-old, single comb White Leghorns hatched from eggs produced at the Minnesota Agricultural Experiment Station. Carefully selected groups were housed in metal, electrically heated battery brooders and maintained on raised screens of coarse mesh. Group food consumptions and individual weights were recorded at weekly intervals and in most experiments the food consumption of the comparative groups was equalized. In all experiments the calcium, phosphorus and nitrogen contents of the comparative rations were also equalized following a preliminary chemical analysis of each feedstuff employed. The rachitogenic property of the rations was judged by the ash content of the left tibia and fibula, after drying and extraction with alcohol and ether.

The rats employed were 28-day-old closely inbred stock reared in this laboratory from mothers fed a modified (higher protein) Steenbock stock diet for production or rats suitable for vitamin D assay. They were housed individually in metal cages on screen floors, care being taken to distribute litter mates as equally as possible among the different experimental groups simultaneously on test. Rachitogenic effect was judged by the ash content of the right and left femurs of each animal, after drying and extraction of lipides.

Nitrogen in feedstuffs was determined by the Kjeldahl method, calcium and phosphorus by the method of Morris, Nelson and Palmer ('31), and water-soluble chloride volumetrically.

Repetition of King-Hall experiments

King and Hall compared the addition of 20 per cent oat groats and oat groats plus vitamin D supplements or carotene to a well-balanced basal ration, approximately 78 per cent of which was corn and wheat. We employed the same basal

ration in a similar study, adding oat groats and also oat groats plus cod liver oil concentrate of known vitamin D potency in comparative tests as shown in table 1. Two additional rations were tested in comparison with the foregoing. These differed from those employed by King and Hall in that oat groats replaced the corn meal in order to secure a higher concentration of oats; the ration of one of these additional groups contained cod liver oil concentrate, the other did not (groups 3 and 6). Table 1 shows the mean bone ash of the various groups after 8 weeks' feeding. The statistical analysis of the data does not indicate that under the conditions employed oat groats was more rachitogenic for chicks than yellow corn meal.

Attempts to concentrate rachitogenic substance

Oatmeal extracts were prepared essentially by the method described by Mirvish ('30), some of the steps being facilitated by semi-plant scale apparatus available in this laboratory. In general the procedure involved 1) two to four successive extractions with 0.5 per cent HCl solutions at room temperatures, 2) one or two successive extractions with hot (80°C. to boiling) 0.5 per cent HCl solution, 3) clarification of the combined extracts by passage through a Sharples supercentrifuge, 4) neutralization with NaOH solution, 5) concentration at reduced pressure, 6) clarification by means of the supercentrifuge, 7) further concentration to a syrup in a warm (70°C.) air chamber, 8) several successive 24- to 48-hour dialyses in cellophane bags against large volumes of distilled water, 9) concentration of the combined diffusates to a viscous mass, 10) repeated extractions of this mass with boiling 95 per cent ethanol, 11) clarification of the combined alcohol extracts by filtration through glass wool.

Four different preparations were made involving some differences in procedure in steps (1) and (2). For example the HCl extractions, first carried out in muslin bags, were later made by suspending the oatmeal directly in the acid and stirring, either continuously by mechanical stirrer for step (1) or intermittently by hand in step (2). The extracts were

TABLE 1

Bone ash of chicks fed rations similar to those employed by King and Hall,[1] with statistical study of data

GROUP	NUMBER OF CHICKS	SUPPLEMENT TO BASAL RATION	BONE ASH		DIFFERENCE BETWEEN MEANS OF GROUPS	P[2]	STATISTICAL SIGNIFICANCE
			Mean S. E.	C. V.			
			per cent	*per cent*			
1	14	1 per cent corn oil	36.4 ± 0.75	7.72			
2	10	20 per cent oat groats plus 1 per cent corn oil	35.5 ± 0.80	7.10	1–2 = 0.9	0.5–0.4	Negative
3	10	20 per cent oat groats replacing 20 per cent corn meal 1 per cent corn oil added	38.2 ± 1.09	9.04	3–1 = 1.8	0.2–0.1	Negative
4	12	0.03 per cent cod liver oil concentrate[3]	44.1 ± 1.59	12.50	4–5 = 3.3	0.2–0.1	Negative
5	14	20 per cent oat groats plus 0.03 per cent cod liver oil concentrate[3]	40.8 ± 1.27	11.63			
6	13	20 per cent oat groats replacing 20 per cent corn meal. 0.03 per cent cod liver oil concentrate[3] added	42.5 ± 1.05	8.93	4–6 = 1.5	0.5–0.4	Negative

[1] King and Hall employed Barred Rocks in their experiments but it seems unlikely that the breed of fowl could be a factor in experiments of this character.

[2] Probability.

[3] The cod liver oil concentrate was diluted with corn oil, the total oil addition being 1 per cent.

syphoned off after settling. In the later extractions boiling was not resorted to; instead porcelain-lined steel drums containing the groats and solvent were immersed in boiling water.

The first two concentrates A and B were found to give positive tests for sugars (Molish test) sterols (faint Whitby-Salkowski test) and basic substances precipitable by phosphotungstic acid. Quantitative analysis showed that 22 to 50 per cent of the original Ca and 2.0 to 5.0 per cent of the original P of the oatmeal had been recovered in these concentrates.

These concentrates and also concentrates C and D, which were prepared by using less than boiling temperatures in step (2), were imposed on rachitogenic rat and chick diets, containing chiefly yellow corn, and their rachitogenic effects tested on these species in comparison with the untreated rations. The amount of concentrate added was equivalent to 200 per cent oatmeal. Similar tests were made with chicks using the material thrown out by the supercentrifuge in each of the two steps in which the centrifuge was employed, as well as the material which failed to dialyze. Data are not presented for the tests on concentrate D using rats and chicks or on any of the other materials recovered for which chicks were employed, since in these tests the preparations proved toxic[2] to the animals, probably because of their high NaCl content.[3] In this respect the results were contrary to those reported by György et al. ('33) who conclude that the high proportion of NaCl resulting from the subsequent neutralization of cereals treated with HCl exerts an anti-rachitic effect when such products are tested biologically.

The results obtained by imposing concentrates A and B on a rachitogenic yellow corn diet for rats and concentrate C on a rachitogenic yellow corn diet for chicks are presented in table 2. The rat tests lasted 21 days and the chick tests 14 days. Extract C was not tested with rats.

[2] The rats lost weight, became highly irritable and savage and died suddenly at an early date. More mature rats behaved similarly but survived longer. The chicks also lost their appetite and only a few survived the experimental period.

[3] Extract D contained 6 per cent NaCl and the chick rations containing the other materials recovered contained from 2.5 to 8.5 per cent NaCl.

TABLE 2

Bone ash of rats and chicks fed rachitogenic corn diets with and without oat concentrate (equivalent to 200 per cent oatmeal) imposed, with statistical analysis

SPECIES	NUMBER OF ANIMALS	RATION	BONE ASH		DIFFERENCE BETWEEN MEANS OF GROUPS	P[2]	STATISTICAL SIGNIFICANCE
			Mean[1] S. E.	C. V.			
			per cent	*per cent*	*per cent*		
Rat	10	Low Ca	46.6 ± 0.79 (1)	5.34			
Rat	8	Low Ca + con. A	43.8 ± 0.60 (0)	3.88	2.8	0.02–0.01	Positive
Rat	9	Med. Ca	26.3 ± 0.95 (1)	10.81			
Rat	9	Med. Ca + con. B	28.2 ± 1.09 (1)	11.58	1.9	0.2 –0.1	Negative
Rat	10	High Ca	27.0 ± 0.75 (0)	8.80			
Rat	10	High Ca + con. B	27.9 ± 0.74 (0)	8.34	0.9	0.4 –0.5	Negative
Chick	33	Basal	37.0 ± 0.40 (7)	6.20			
Chick	13	Basal + con. C	36.6 ± 0.71 (2)	2.88	0.4	0.6 –0.5	Negative

[1] Based on surviving animals, mortality being shown in parenthesis.

[2] Probability.

In the study of concentrate A, using rats, the basal rachitogenic diets were in part modifications of the Steenbock-Black ('24) diet, only 1 or 2 per cent $CaCO_3$ being added respectively in two of the diets instead of the prescribed 3 per cent, the purpose of the modifications being to produce several different degrees of rickets in order to bring out more clearly the presence of rachitogenic substances in the concentrate. The modified rations thus consisted of $CaCO_3$ 1, 2 or 3 parts, NaCl 1 part, commercial wheat gluten 20 parts and yellow corn to make 100 parts. The lowest Ca ration contained approximately 0.41 per cent Ca and 0.34 per cent P. When the oat extract A was imposed on it these values increased to 0.48 and 0.35 per cent Ca and P, respectively.

The rachitogenic ration given the chicks was a modification of the Hart-Kline-Keenan ('31) ration consisting of yellow corn 70 parts, wheat flour middlings 10 parts, crude domestic casein 12 parts, alfalfa leaf meal 3 parts, whole dried yeast 2 parts, $Ca_3(PO_4)_2$ 1 part, $CaCO_3$ 1 part, NaCl 1 part. This ration contained 3.05 per cent N, 0.94 per cent Na and 0.78 per cent P. When the oat extract C was imposed the yellow corn was reduced to 64 parts, the $Ca_3(PO_4)_2$ reduced to 1.54 parts and the $CaCO_3$ reduced to 0.74 parts. This ration contained 2.92 per cent N, 0.85 per cent Ca and 0.68 per cent P, essentially the same as the basal ration. The rations were also essentially isodynamic. The group food consumption of the chicks in the two lots was also equalized by keeping close record of the daily food consumption. The mortality was seven out of twenty birds in the group fed the oat extract.

The data in table 2 show that oats concentrate A was definitely rachitogenic for the rats when imposed on the Steenbock-Black diet containing only 1 per cent added $CaCO_3$ but that concentrate B did not show this effect when imposed on a like ration containing higher concentrations of Ca. Also, concentrate C was without effect when imposed on the rachitogenic chick ration. Unfortunately there was not sufficient of the concentrate C to repeat the rat experiment using the low Ca diet. It is possible that the milder heat treatment

employed for the preparation of this concentrate failed to secure the rachitogenic substance or that the different lot of oatmeal employed lacked the principle. Of the two explanations the former appears more plausible in view of other experiments with chicks in which this lot of oatmeal was more rachitogenic than corn. The possibility of chicks being less susceptible to rachitogenic substance must be discounted in view of the results presented below.

Consideration of the rat data alone suggests that if concentrate B, which was made almost exactly like concentrate A, contained the active principle it probably could not be expected to lower still further the ash of bones already rendered so strongly rachitic by the basal ration. There should be a limit to the severity of decalcification (of lack of calcification) caused by a rachitogenic diet. It is unfortunate that insufficient material prevented a test of the effect of concentrate B on chicks and on rats fed the low Ca ration (containing 1 per cent $CaCO_3$).

The suggestion that a rachitogenic principle becomes evident in these types of experiments only when the basal ration is itself only mildly rickets producing is strengthened by the following experiments conducted on chicks.

Relation of severity of rickets to demonstration of rachitogenic principle

The modification of Hart-Kline-Keenan ('31) rachitogenic ration for chicks, described above, gives satisfactory growth and calcification when fortified with adequate vitamin D, but alone ordinarily produces severe rickets in young chicks in the course of a few weeks. Since this ration contains 70 per cent yellow corn it would seem to be ideal for comparison of rachitogenic properties of different cereals which may be substituted in toto for the yellow corn. Moreover, protein (casein), vitamin (yeast and alfalfa leaf meal) and mineral ($(Ca_3PO_4)_2$ and $CaCO_3$) additions make it possible to adjust the N, Ca and P contents of such comparative cereal rations so that no known major quantitative differences exist between

TABLE 3

Bone ash of chicks fed yellow corn vs. rolled oats rachitogenic rations and statistical significance of results

EXPERIMENT NO.	DATE OF EXPERIMENT	NUMBER OF CHICKS	RATION	LENGTH OF EXPERIMENT	BONE ASH		DIFFERENCE BETWEEN MEAN ASH	P[4]	STATISTICAL SIGNIFICANCE
					Mean[1] S. E.	C. V.			
				weeks	*per cent*	*per cent*	*per cent*		
1	Aug. 1932	35	Yellow corn	3	36.9 ± 0.42(3)	6.45			
1	Aug. 1932	34	Rolled oats	3	31.0 ± 0.61(10)	9.68	— 5.9	Less than 0.01	Positive
2	Nov. 1932	13	Yellow corn	2	33.3 ± 0.61(0)	6.59			
2	Nov. 1932	10	Rolled oats	2	31.5 ± 0.89(0)	8.90	— 1.8	0.1	Negative
3a	Feb. 1933	14	Yellow corn	2	29.3 ± 0.82(4)[2]	8.79			
3a	Feb. 1933	21	Rolled oats	2	28.3 ± 0.71(11)[2]	7.90	— 1.0	0.6–0.7	Negative
3b	Feb. 1933	14	Yellow corn	3	29.7 ± 0.78(3)[3]	8.67			
3b	Feb. 1933	14	Rolled oats	3	28.9 ± 1.15(6)[3]	11.27	— 0.8	0.5–0.6	Negative
4	May 1933	35	Yellow corn	2	37.0 ± 0.40(2)	6.20			
4	May 1933	47	Rolled oats	2	32.0 ± 0.38(6)	7.64	— 5.0	Less than 0.01	Positive

[1] Based on chicks which survived, the mortality being shown in parenthesis.
[2] Birds dying during first 2 weeks.
[3] Birds dying during third week.
[4] Probability.

them. Besides, such qualitative differences in vitamins A and B (complex) as do exist cannot be regarded as rendering any of them inadequate in these respects.

On the above basis several comparisons were made between rations containing 70 per cent yellow corn meal and 70 per cent rolled oats. Not all of these comparisons were made at the same time although experiments 1, 2 and 3 (a and b) were carried out using rations made at a single mixing. The rations for experiment 4 were mixed at a later date. The results are shown in table 3. Experiments 3a and 3b are the same experiment, one-half of the birds in each group being sacrificed at the end of 2 weeks, the remainder being continued to the end of the third week. In each experiment the feed consumption was equalized for each two comparative groups.

The data seem to furnish strong support for several suppositions. First, there is a marked seasonal difference in the susceptibility of chicks to rickets. A further discussion of this difference has been given by Lachat ('34). Second, a greater rachitogenic property of oats is demonstrable when the degree of rickets is mild, and (or) the susceptibility less. Third, when the rachitic condition is extreme a further concentration of the factors causing it does not produce a still further lowering of the bone ash. Fourth, the degree of rickets produced in the first and fourth experiment with yellow corn being the same and also like that in the test for oat concentrate C reported above, which was conducted simultaneously with the fourth experiment, this concentrate evidently did possess rachitogenic substances.

CONCLUSIONS[4]

1. Hydrochloric acid extracts of oats, when purified and freed from excessive amounts of NaCl, may exhibit rachitogenic properties when fed to rats in a mildly rachitogenic ration, but not when the ration is severely rachitogenic.

[4] The conclusions are taken essentially verbatim from Doctor Lachat's thesis.

2. Rolled oats and yellow corn are rachitogenic to both rats and chicks, especially to the latter, and rolled oats appears to be more rachitogenic when the rations are otherwise only mildly rickets producing or the susceptibility of the animals is low.

3. Divergent results obtained by others in the study of the relative rachitogenic properties of oats and other cereals probably may be explained by lack of control of the severity of the rickets produced by the ration with which the cereal rations have been compared.

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CORRECTION

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Page 513, line 18. Sentence beginning "When half of the inulin of diet 11" should read "When the amount of inulin present in diet 11 was added as a supplement to the basal sucrose-containing diet (diet 10 a) all the animals grew well, an average gain per rat of 36.3 gm. being recorded."